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GONADOTROPIC ACTIVITY OF THE PITUITARY GLAND IN RELATION TO THE SEASONAL SEXUAL CYCLE OF THE COTTONTAIL RABBIT (SYLVILAGUS FLORIDANUS MEARNSI)[1]

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FOUR FIGURES

The purpose of this work was to investigate the reproductive cycle of a non-hibernating wild mammal. The cottontail rabbit (Sylvilagus floridanus mearnsi Allen) was selected for this study because it is an abundant species of wide occurrence and of great economic importance. The weight changes in the endocrine glands directly related to the breeding cycle and the changes in the accessory reproductive organs of both male and female cottontail rabbits have been followed throughout the year to determine the relationship which exists between the degree of development of the reproductive tract and the gonadotropic content and percentage of cell types in the pituitary gland. The endocrine factors in the sexual cycle of a hibernating wild mammal, the thirteen-lined ground squirrel, have been thoroughly investigated, but complete results have been published only for the male sex (Wells, '35 and '38; Moore et al., '34; Nelson, '36). Brief notes on the measurements of the gonads of cottontail rabbits have been recorded by Hamilton ('40), Dalke ('37), and Trippensee

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('36). The latter also weighed the testes during the seasons in which the male rabbits were sexually active and inactive. Recently Schwartz ('42) has made monthly measurements of the ovaries and testes, but reported no weights. The duration of the nesting period has been studied by Beule and Studholme ('42).

MATERIAL AND METHODS

The rabbits were secured from the University of Wisconsin arboretum by box-trapping in the winter and shooting in the summer. The pituitary glands of animals shot in the field were removed immediately, inserted in a vial and the vial put in a thermos bottle filled with cracked ice and then transferred to a freezing unit upon return to the laboratory. The carcasses were brought to the laboratory for complete autopsy. All animals secured by box-trapping were brought to the laboratory alive and killed by a blow on the head or by asphyxiation with illuminating gas. The pituitary glands were removed, weighed and frozen. The testes, the seminal vesicles and prostate glands with their secretions, the ovaries, and uteri were removed, weighed and preserved in Bouin's fluid. Sperm smears were made from the epididymides of all males showing testicular stimulation.

The pituitary glands from each sex at each month were pooled and assayed for their gonadotropic hormone content. Glands were not stored for longer than 1 month because Kupperman, Elder and Meyer ('41) have shown that prolonged storage of frozen pituitary glands causes loss in gonadotropic activity while storage for 35 days causes no significant loss.

The frozen glands were prepared for assay by triturating them in a cone-shaped centrifuge tube, adding a few drops of water, and flushing through a small bore hypodermic needle until a fine suspension resulted. After the suspension was diluted so that 4.5 cc. was equal to 30 mg. equivalent of fresh tissue, it was assayed in 21-day old female white rats. The afternoon of the first day 0.5 cc. was injected subcutaneously and the same dose administered twice daily for the next 4 days. The animals were autopsied on the morning of the sixth day. During the early months of the study part of each group of glands was assayed with heme as an augmenter as described by McShan and Meyer ('41), and part without heme. The addition of this augmenter made possible the assay of smaller amounts of material than otherwise would have been possible, especially during the anestrous season when pituitary gonadotropic activity is low. Each quantity of pituitary gland suspension was mixed in vitro with heme which was prepared by dissolving 4 mg. of hemin in dilute sodium carbonate solution. All assays were made by injecting a total dose equivalent to 30 mg. of fresh rabbit glands into each rat. The increase in weight and degree of luteinization of the ovaries of the recipient rats was used as a quantitative measurement of gonadotropic activity.

The pituitary glands of eleven male and twelve female rabbits were preserved in Bouin's fluid, imbedded, sectioned at 6 micra and stained with a modified Mallory's trichrome stain. All the cells in every tenth microscopic field of a medial horizontal section were differentially counted at a magnification of 1350. In each field which was examined, the acidophiles were counted first, then the basophiles, and finally the total number of cells. From these figures the percentage of each cell type was calculated.

SEASONAL CHANGES IN THE PITUITARY GLAND AND REPRODUCTIVE SYSTEM OF THE MALE COTTONTAIL RABBIT

The quiescent period of the reproductive tract of the male cottontail rabbit is 4 to 5 months, September to December inclusive. The first signs of stimulation of the reproductive tract appear in late December. This has been described by Schwartz ('42), and was also reported by Dalke ('37), Hamilton ('40), and Trippensee ('36). This is shown by an increase in size and weight of the testes, which is apparent in nearly all animals in January and by February the average testicular weight is approximately 12 gm. or about one-half the maximum average of 23 gm. found in May. The weight of the testes at the peak of stimulation is approximately 27 times the average weight of 0.8 gm. found in September. Figures 1 and 2 show monthly averages resulting from plotting the data representing 125 adult male testes, prostate gland,

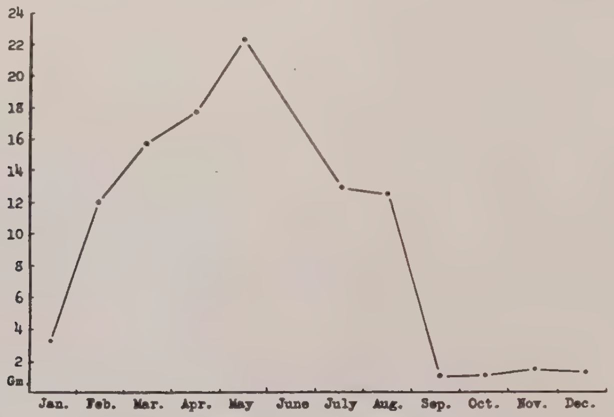

Fig. 1 Monthly average weight of the testes of cottontail rabbits.

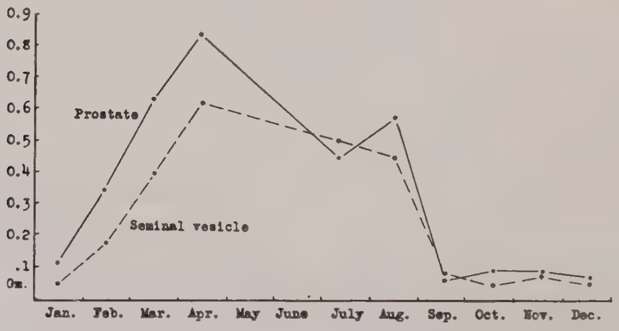

Fig. 2 Monthly average weight of the prostate gland and seminal vesicles of cottontail rabbits.

and seminal vesicle weights. Each point represents the average organ weight for a particular month. The average weights of testes, seminal vesicles, and prostate glands for each month are shown in table 1. The curves show that the increase in testes weight slightly precedes that of the accessories, for by February the average testicular weight is one-half the maximum while the average accessory gland weight is only one-third the maximum weight that it will later attain. During the remainder of the active season the curves of testes, prostate, and seminal vesicle weights are similar in shape and all decline sharply as the entire reproductive tract rapidly regresses in August and reaches the inactive level in September. This is very different from the condition which Wells ('35) found in the ground squirrel. In this species the testes regressed rapidly before the accessory reproductive organs had reached their maximum stimulation.

TABLE 1

Weights of endocrine glands and accessory reproductive organs of cottontail rabbits.

MONTH	NO. OF RABBITS	AVERAGE PITUITARY WEIGHT	AVERAGE TESTES WEIGHT	AVERAGE PROSTATE WEIGHT	AVERAGE SEM. VES. WEIGHT	AVERAGE OVARY WEIGHT
		mg.	*mg.*	*mg.*	*mg.*	*mg.*
January	♂ 26	12.5	3.1	110	45	
	♀ 27	13.7				74
February	♂ 23	14.0	11.8	344	175	
	♀ 12	13.7				87
March	♂ 35	15.1	15.5	621	387	
	♀ 7	19.3				113
April	♂ 15	16.8	17.5	831	612	
	♀ 12	17.6				135
May	♂ 3	17.5	22.0	675	506	
	♀ 1	24.0				175
June	♂ 1	10.0	15.4	602	825	
	♀ 2	24.0				191
July	♂ 4	15.5	12.7	438	493	
	♀ 5	20.4				172
August	♂ 4	15.5	12.2	565	446	
	♀ 2	27.7				163
September	♂ 4	13.5	0.8	61	86	
	♀ 5	19.6				77
October	♂ 6	14.4	0.8	89	44	
	♀ 10	19.5				87
November	♂ 6	13.3	1.2	89	76	
	♀ 12	15.0				68
December	♂ 17	13.8	1.0	72	48	
	♀ 17	14.7				68

The disappearance of sperm from the epididymis occurs during the same month as the rapid regression in the weight of the testes, prostate, and seminal vesicles. August is the month of cessation of male reproductive activity in the region of Madison, Wisconsin. This is similar to Trippensee's findings in Michigan, but in New York State, Hamilton ('40) observed that males were active at least a month longer, a few having "enlarged testes in October." However, neither Trippensee nor Hamilton report any observations on the presence of spermatozoa in the epididymis during the period of regression.

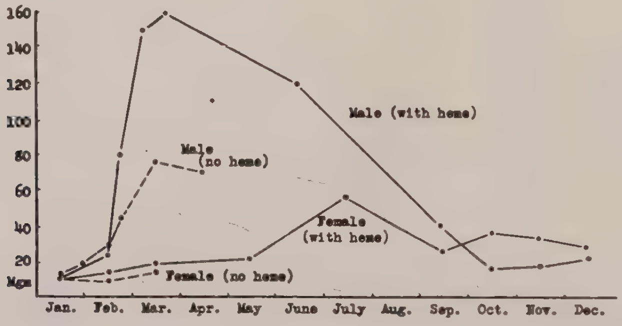

Fig. 3 Gonad-stimulating activity of cottontail rabbit pituitary glands as measured by the ovarian weights of immature rats.

Seasonal changes in the gonadotropic potency per unit weight of pituitary tissue are shown in figure 3. Table 2 gives the exact dates during which each group of glands was collected for assay. The number of test animals depended upon the abundance of rabbits available as pituitary donors in each month. All assays of the male rabbit pituitary glands from May through August were given as one average, for all donors were in the same physiological state so far as the degree of stimulation of the reproductive tract was concerned. The point representing this average was placed on the graph midway between May and August (fig. 3).

It seems clear that the gonadotropic activity of the pituitary gland of male cottontail rabbits reaches a minimum in October and remains at this low level for 4 months, through January. A slight increase in the potency of the male rabbit pituitary gland in February is followed by a rapid and tremendous increase of 600% in March. This high level is maintained throughout the summer but decreases rapidly in early fall and again reaches a minimum in October.

The period of greatest weight of the pituitary gland is roughly the same as that of increased gonadotropic activity.

TABLE 2

Gonadotropic hormone content of cottontail rabbit pituitary glands.

MALE RABBITS				FEMALE RABBITS			
Period of collection of donor rabbits	No. of rats	Average ovarian weight without heme	Average ovarian weight with heme	Period of collection of donor rabbits	No. of rats	Average ovarian weight without heme	Average ovarian weight with heme
		mg.	*mg.*			*mg.*	*mg.*
January 5–28	2	14	14	January	9	13	18
Jan. 27–Feb. 2	1	20					
February 9–28	3	29	24	February	3	9	13
February 15–25	2	45	81				
March	3	75	149	March	7	13	21
April	4	71	160				
May 1–Aug. 31	5		138				
September	2		42	Sept. 25–30	3		28
October	2		16	October	4		36
November	7		17	November	3		34
December	3	13	22	December	3	17	31

All rats received 30 mg. fresh weight of rabbit pituitary tissue.

There is no sharp increase in weight paralleling the rapid increase in activity with the onset of the breeding season, nor sharp decrease with the rapid decline in activity of the gland in late summer.

Whenever there is an increase in the gonad stimulating activity of the male rabbit pituitary gland it is at once reflected in the degree of development of the reproductive tract. Conversely when the gonadotropic hormone content is decreasing the reproductive tract is regressing. Therefore, it would seem that the anestrous season is due to a failure of the pituitary gland to produce adequate amounts of gonadotropic hormone, and not the result of any failure to liberate the hormone.

For the purpose of discovering whether any changes occur in the cellular constitution of the pituitary gland from estrous to anestrous seasons which might be correlated with the observed changes in gonadotropic content of the gland, microscopic studies were made of the glands of five male rabbits obtained in December, the month of least sexual development, and those of six male rabbits obtained in March, the month of greatest sexual development.

TABLE 3

Cell type analysis and relative gonadotropic content of pituitary glands in relation to seasonal reproductive activity of the cottontail rabbit.

SEASON	SEX	AVERAGE GONAD WEIGHT	PITUITARY ASSAY DATA		PITUITARY CELL TYPE ANALYSIS			
			No. of rats	Average ovarian weight	No. of rabbits	% Acid.	% Baso.	% Chrom.
				mg.				
Anestrous (December)	Male	1.0 gm.	3	22	5	27.8	4.4	67.8
Estrous (March)	Male	15.5 gm.	3	148	6	20.8	13.8	65.4
Anestrous (December)	Female	58.0 mg.	3	31	10	20.9	6.6	72.5
Estrous (March)	Female	113.0 mg.	7	21	2	24.7	3.9	71.4

In both seasons there is a marked correlation between the percentage of basophilic cells, the gonadotropic content of the pituitary gland and the degree of development of the gonads and reproductive tract (table 3). During the estrous season the gonadotropic content of the pituitary glands of male rabbits is six times greater than during the anestrous season. At this same period there is a three-fold increase in percentage of basophile cells and a fifteen-fold increase in weight of the testes. In December the percentage of basophiles was 4.4% and the average weight of the testes was 1.0 gm. At the height of the breeding season, in March, the percentage of basophiles was 13.8 and the average weight of the testes was 15.5 gm. Biological assay of the pituitary glands of all the male rabbits recorded in December gave an average ovarian weight in the immature female rat recipients of 22 mg., whereas the glands of those obtained in March gave an average ovarian weight of 148 mg. The increased conversion of chromophobes to basophiles is reflected in the number of cells per microscopic field. When only 4.4% of the cells were large basophiles, there was an average of 145 cells per microscopic field, while during the estrous season, when 13.8% of the cells were large basophiles, an average of only ninety-six cells was found in each microscopic field. The reduction in number of cells per field seems to be proportional to the degree of cellular hypertrophy and to the gonadotropic content of the pituitary gland.

SEASONAL CHANGES IN THE PITUITARY GLAND AND REPRODUCTIVE SYSTEM OF THE FEMALE COTTONTAIL RABBIT

Ovarian weight alone was not used as a criterion in determining the duration of the active and quiescent seasons of the reproductive activity of the female cottontail rabbit. An examination of figure 4 will show that there is not as sharp a demarcation of estrous and anestrous seasons indicated by monthly average ovarian weights as was seen in the monthly average testicular weights in figure 1. This, in a great part,

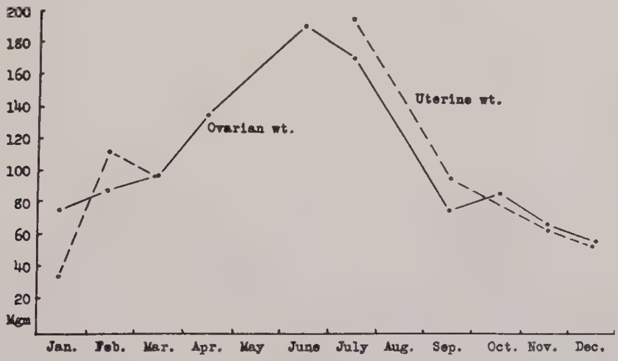

Fig. 4 Monthly average weight of the ovaries and uterus of cottontail rabbits. (Each unit on the graph represents 20 decigrams of uterine weight.)

is due to the fact that the percentage increase in ovarian weight at the time of the breeding season is much less than that of the testes. Since the minimum monthly average weight of the ovary is 58 mg. and the maximum average weight 191 mg. (table 1), there is an increase in weight of only 3.3 times from the quiescent to the active breeding season. In the male the increase in average testicular weight is from 0.8 gm. to 22 gm., or an increase of 27 times in weight from the quiescent to the active season. This weight increment being 9 times that of the ovary, the curve of ovarian weights is necessarily flat compared with that of the weights of the testes and does not show clearly the limits of the breeding season. This conforms with the comparatively small changes in the amount of gonadotropic hormone in the pituitary gland of the female. The sharp demarcation between the estrous and anestrous seasons shown by Schwartz' ('42) curves of ovarian volume is probably due to the fact that he did not determine actual volume by displacement, but by computation. Thus corpora lutea projecting from the smooth surface of the rabbit ovary would greatly increase the apparent volume computed by multiplying length by width by thickness.

The uterus was found to be very sensitive to increased ovarian activity, and showed definite stimulation with increased ovarian weight at the end of January. Figure 4 shows a partial curve of uterine weights. No weights of pregnant uteri were recorded because of the obvious difficulty in eliminating placental material. The available portion of the curve of average uterine weights shows a close parallel with the ovarian weight curve at that season — a slow decline to a base level reached in December. It is during this time that placental sites in the uterus remain discernible and can be used as an age criterion (Elder and Sowls, '42). This is based on the finding of a 100% incidence of pregnancy. April 4th is the latest date on which a non-pregnant female was taken in any of the three breeding seasons during which the study was made.

The earliest ovulation occurred March 15th as determined by the presence of ovulation papillae and eggs in the uteri. The eggs were found by flushing out the contents of the oviducts and uteri with normal saline. The washings were examined under a binocular microscope for the presence or absence of ova. The latest ovulation determined by these methods was August 10th. This establishes the minimum duration of the breeding season as approximately 5 months — from mid-March to mid-August — which correlates in general with the duration of spermatogenesis in the male. However, the earliest ovulation observed was 5 weeks after spermatozoa were first found in epididymal smears. Thus, while the onset of the breeding season seems to be controlled by the female, the termination is not controlled by one sex alone, for both males and females become sexually inactive at the same time.

The potency of female rabbit hypophyseal tissue shows but little change through the year, neither paralleling the sharp increase in potency of the male gland with the onset of the breeding season nor the extreme decrease in activity exhibited by the male gland in the fall. The average weight of the rat ovary secured by the assay of pituitary glands of post partum female cottontail rabbits was placed on the graph (fig. 3) as if occurring in mid-July. Greatly increased potency in the female rabbit pituitary gland was found only in those post partum animals not yet pregnant a second time. This material gave an average weight for the rat ovary of 57 mg. or approximately twice that resulting from the injection of female rabbit pituitary glands at any other time. An attempt was made to discover whether or not any change in amount occurred as pregnancy progressed, but the data in table 4 show that no significant difference obtained. Therefore, all rat ovarian weights secured from assay of pregnant rabbit pituitary glands were averaged (24 mg.) and the point placed, arbitrarily, on the graph as if occurring in May (fig. 3).

It is of interest that the average weight of the female hypophysis exceeds that of the male even during the breeding season when the gonadotropic potency per milligram of tissue is but 12% of that of the male.

The results of the microscopic studies of female rabbit hypophyses are difficult to explain because the changes between seasons are less marked and the gonadotropic content of the pituitary gland is greater during the anestrous season when the ovaries and reproductive tract are less developed. However, in the female rabbits the percentage of basophiles appears to be proportional to the gonadotropic content. In December the average ovarian weight is 58.0 mg., while in March they have an average weight of 113.0 mg. The percentage of basophiles in December is 6.6% in contrast to 3.9% in March, which correlates with the ovarian weight obtained in recipient female rats by biological assay of the glands from these 2 months, of 31 mg. in December and 21 mg. in March.

TABLE 4

Gonadotropic hormone content of pituitary glands of pregnant and post partum cottontail rabbits.

TYPE OF RABBIT	NO. OF RATS	AVERAGE OVARIAN WEIGHT
		mg.
Pregnant — all stages, ungrouped	5	24
Pregnant — post ovulatory, prior to implantation	3	25
Pregnant — embryos less than 20 mm. long	3	22
Pregnant — embryos more than 20 mm. long	2	28
Pregnant — total of all types	13	24
Post-partum	3	57

DISCUSSION

The seasonal rhythm of reproductive activity in the male cottontail rabbit is similar to that which has been described for the ground squirrel (Wells, '36; Moore, et al., '34) and the woodchuck (Rasmussen, '18). These previous reports were concerned with hibernating species of wild mammals which were thus more or less isolated from environmental influences. Since the cottontail rabbit is a species which does not hibernate, its sexual cycle is possibly more definitely associated with seasonal changes in the environment. The sexually active period of the cottontail rabbit has a duration of approximately 8 months, from February to September, followed by a quiescent period of 4 months, during which marked regression of the reproductive system occurs.

In the male cottontail rabbit this seasonal development in the reproductive system is correlated with changes in the gonad stimulating activity of the pituitary gland as shown by biological assays. Cytological studies indicate that this is accompanied by an increased percentage of basophilic cells in the pars distalis of the pituitary gland. The increased gonadotropic activity of the glands during times when high percentages of basophiles are found is correlated with the presence of secretion granules in their cytoplasm.

The cause of this cytological change in the pituitary gland is problematical, but may be associated in some way with an inherent glandular rhythm or with the effect of external environmental factors, such as temperature or length of daylight. Within a few weeks following the winter solstice the first increase in pituitary gland activity (fig. 3) was detected by assay, and was also reflected in the onset of testicular hypertrophy (fig. 1).

The fact that the gonadotropic content of hypophyseal tissue of the male cottontail rabbit exceeds that of the female throughout the 8 months of the year during which the male gland is not at base level is suggestive of the condition reported in the domestic rabbit. Saxton and Green ('39) using guinea pig ovarian weight as an end-point, Smith, Severinghaus and Leonard ('33) making implants of pituitary tissue in immature mice, and Bergman and Turner ('42) using increase in gonad weight of baby chicks as a measure of gonadotropic activity, all agree that the hypophyseal tissue of the male domestic rabbit exceeds that of the female. However, Hill ('34) in an earlier paper found acetone-dried glands of female domestic rabbits to have more gonadotropic activity than those of males.

The ovarian weight of the female cottontail rabbit increases three-fold during the breeding season, although there is a decrease in both the gonadotropic hormone content and the percentage of basophilic cells. This makes the control of the breeding season in the female more difficult to understand. Follicular development and ovarian secretion do occur even though there is an apparent reduction in gonadotropic activity at the onset of the estrous period. This in indicated by the growth of follicles, uterine stimulation, ovulation papillae and recovery of ova from the oviduct. The fact that the highest ovarian weight is recorded in mid-summer suggests that luteinization is responsible, to a large extent, for the shape of the curve of ovarian weights (fig. 4).

It has been shown that isolated female domestic rabbits undergo follicular development and remain in a state of almost continuous "heat." If the reproductive cycle of the female cottontail rabbit during the breeding season is similar to that of the domestic rabbit, it would seem possible that the basophilic cells produce FSH which keeps the rabbit in "heat" until copulation and ovulation occur. This may be accompanied by increased formation of acidophilic cells which are thought to produce LH, since Friedgood and Dawson ('38) describe an increase in the "carmine cell," a type of acidophile, when ovulation is induced in domestic rabbits.

SUMMARY

A study has been made of wild cottontail rabbits for the purpose of determining the changes which occur in the pituitary gland and the reproductive system throughout the year.

The gonadotropic activity of the male pituitary gland remains at a minimum for 4 months, October through January. This is followed by a rapid increase in activity which reaches a maximum of 600% in March. This high level is maintained through the summer, decreases rapidly in early fall, and by October has reached the inactive level.

The percentage of basophilic cells in the pituitary gland of the male increases from 4.4% in the inactive season to

13.8% during the breeding season. This is accompanied by a slight decrease in percentage of acidophiles.

Curves representing the average monthly weights of the testes, seminal vesicles and prostate glands show the same abrupt rise with the onset of the breeding season as does the curve representing hypophyseal activity. All curves remain similar in shape and decline sharply as the entire reproductive system rapidly regresses in August.

The female cottontail rabbit, in which the ovarian weight increases three-fold during the breeding season, undergoes no significant fluctuation in either gonadotropic potency or percentage of basophilic cells of the pituitary gland. The breeding season was found to have a duration of 5 months, mid-March to mid-August, as determined by uterine stimulation, ovulation papillae and presence of eggs in the oviduct. This correlates with the duration of spermatogenesis in most males. An increased gonadotropic activity (100%) of the pituitary gland of the female was found only in post partum animals not yet pregnant a second time.

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THE RELATIONS OF THE INFERIOR LARYNGEAL NERVE TO THE INFERIOR THYROID ARTERY

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ONE FIGURE

In spite of the importance of the anatomical relationship of the inferior laryngeal nerve to the inferior thyroid artery, in the surgery of the thyroid gland, only one paper (Fowler and Hanson, '29) containing a sizable collection of data on this subject has been located in the literature. Most anatomy text books state merely that the inferior laryngeal nerve may pass either superficial or deep to the inferior thyroid artery. It is the purpose of this paper to present the observations made during the dissection of 506 inferior laryngeal nerves (253 cadavers).

MATERIAL AND METHODS

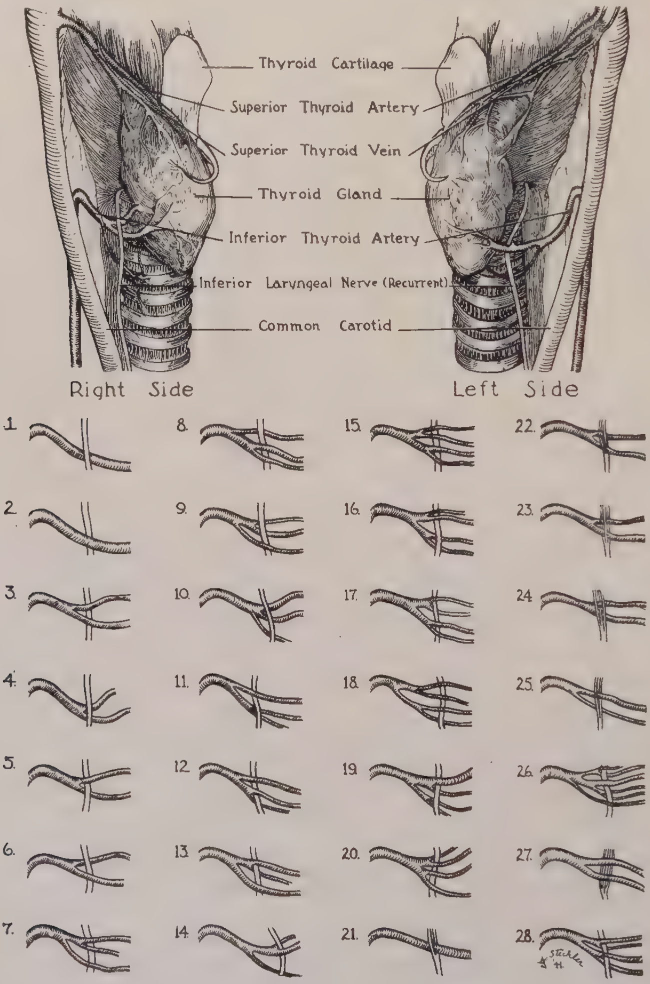
During the routine dissection of 253 cadavers in the Gross Anatomy Laboratory at Tulane records were made of the relations of the inferior laryngeal nerve to the inferior thyroid artery. The only selection of material was the elimination of those bodies in which accurate comparison of the right and left sides could not be made, owing to the fact that branches of the inferior thyroid artery had been broken on one side.

The frequencies of the types of relations were separately tabulated for right and left sides. The types were further combined into groups in which the nerve passed superficial to the artery or its branches, deep to the artery or its branches, and between the branches of the artery. Variations in the branches of the nerve and developmental anomalies were also combined in groups.

OBSERVATIONS

Twenty-eight types of relations were found (fig. 1). The occurrences of these different types are listed in table 1. The result of grouping the related individual types into classes is seen in table 2.

In addition to the twenty-eight types three right inferior laryngeal nerves were distinguished by unusual courses associated with variations in the right subclavian arteries.


Thyroid Cartilage
Superior Thyroid Artery
Superior Thyroid Vein
Thyroid Gland
Inferior Thyroid Artery
Inferior Laryngeal Nerve (Recurrent)
Common Carotid
Right Side
Left Side
1.
2.
3.
4.
5.
6.
7.
8.
9.
10.
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28.

Fig. 1 The types of relations of the inferior laryngeal nerve to the inferior thyroid artery.

TABLE 1

Types of relations.

(Refer to fig. 1 for types listed in "right" and "left" columns.)

BODY	RIGHT	LEFT	BODY	RIGHT	LEFT	BODY	RIGHT	LEFT	BODY	RIGHT	LEFT
1	10	5	65	3	4	128	1	19	191	3	3
2	1	2	66	11	15	129	5	2	192	3	3
3	24	18	67	4	3	130	2	11	193	11	2
4	1	2	68	21	10	131	7	2	194	10	14
5	6	2	69	11	4	132	2	21	195	11	2
6	11	5	70	11	10	133	2	6	196	1	1
7	24	2	71	4	12	134	2	2	197	2	2
8	25	18	72	6	6	135	2	2	198	4	15
9	5	12	73	4	16	136	2	1	199	1	5
10	25	26	74	18	12	137	2	2	200	2	2
11	1	3	75	21	7	138	3	4	201	4	2
12	5	6	76	12	7	139	2	4	202	5	4
13	1	2	77	11	7	140	2	5	203	2	2
14	1	6	78	3	3	141	a.n.[1]	5	204	5	3
15	19	20	79	21	4	142	13	20	205	1	5
16	7	27	80	2	3	143	3	1	206	1	5
17	11	2	81	5	5	144	2	3	207	1	2
18	5	3	82	4	3	145	19	1	208	5	5
19	13	21	83	3	3	146	2	5	209	1	6
20	2	2	84	5	3	147	11	2	210	9	23
21	5	2	85	11	11	148	3	7	211	17	3
22	2	8	86	12	7	149	21	3	212	11	3
23	15	3	87	3	3	150	6	3	213	16	2
24	14	2	88	11	3	151	2	2	214	7	3
25	1	5	89	12	7	152	2	2	215	22	21
26	2	2	90	5	4	153	1	2	216	5	12
27	1	5	91	3	5	154	2	2	217	3	8
28	27	14	92	11	4	155	4	3	218	1	1
29	3	7	93	12	3	156	5	2	219	22	13
30	6	6	94	5	16	157	5	3	220	2	1
31	7	2	95	3	12	158	5	6	221	2	13
32	1	5	96	8	4	159	20	14	222	5	3
33	2	2	97	4	7	160	5	6	223	5	1
34	2	15	98	7	11	161	1	2	224	4	2
35	3	14	99	5	3	162	4	2	225	5	3
36	12	5	100	3	11	163	1	5	226	1	7
37	a.n.[1]	5	101	3	5	164	3	3	227	21	5
38	6	5	102	11	3	165	3	7	228	13	6
39	16	2	103	22	3	166	5	4	229	22	22
40	5	5	104	4	4	167	12	12	230	16	3
41	1	11	105	5	3	168	5	2	231	1	2
42	8	6	106	15	3	169	1	3	232	6	7
43	16	19	107	16	10	170	11	5	233	5	5
44	7	1	108	6	3	171	5	3	234	1	1
45	3	3	109	5	6	172	21	5	235	4	2
46	1	3	110	11	5	173	1	2	236	1	2
47	1	2	111	3	7	174	5	1	237	5	5
48	5	28	112	6	3	175	5	5	238	13	13
49	4	2	113	5	3	176	2	2	239	3	14
50	12	11	114	1	3	177	6	5	240	5	2
51	27	2	115	12	3	178	5	3	241	1	5
52	4	5	116	4	6	179	a.n.[1]	12	242	2	13
53	4	3	117	4	15	180	5	5	243	1	2
54	2	2	118	4	7	181	4	9	244	3	3
55	12	8	119	3	4	182	22	2	245	1	6
56	1	7	120	15	3	183	2	3	246	21	6
57	1	5	121	5	3	184	5	3	247	3	5
58	1	3	122	3	3	185	3	3	248	5	5
59	7	7	123	4	4	186	21	16	249	1	3
60	1	3	124	4	3	187	4	4	250	5	3
61	1	6	125	12	17	188	4	4	251	5	2
62	2	6	126	3	7	189	4	3	252	25	3
63	11	7	127	3	2	190	21	3	253	5	4
64	4	2									

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[1] Anomalous inferior laryngeal nerve.

TABLE 2

Groups of related individual types.

TYPES	RIGHT SIDE		LEFT SIDE		BOTH SIDES
	Absolute number	Per cent	Absolute number	Per cent	Per cent
Nerve anterior to the artery or its branches: types 1, 4, 10....	65	25.7	29	11.5	18.6
Nerve posterior to the artery or its branches: types 2, 3, 7, 15..	68	26.9	130	51.4	39.1
Nerve anterior to one half and posterior to one half of the branches of the artery: types 5, 6, 16, 26, 28	56	22.1	55	21.7	21.9
Nerve anterior to one third and posterior to two thirds of the branches of the artery: types 8, 11, 12, 13	33	13.0	20	7.9	10.5
Nerve anterior to two thirds and posterior to one third of the branches of the artery: types 9, 14	2	0.8	6	2.4	1.9
Nerve anterior to one fourth and posterior to three fourths of the branches of the artery: types 17, 18, 20	3	1.2	5	2.0	1.9
Nerve anterior to three fourths and posterior to one fourth of the branches of the artery: type 19	2	0.8	2	0.8	0.9
Nerve divided into two branches while in direct relation to the artery or its branches: types 21, 22, 25	17	6.7	4	1.6	4.2
Nerve divided into three branches while in direct relation to the artery or its branches: types 23, 24, 27	4	1.6	2	0.8	1.2
Anomalous nerve	3	1.2			0.6
Relations of the nerve to the artery alike on the two sides..					17.0

These three nerves were quite similar in course and relations. Each arose from the vagus at about the level of the larynx, passed downward and medially behind the common carotid and inferior thyroid arteries, giving off the usual branches, and entered the larynx. In each case no innominate artery was present; the right subclavian artery arose from the left part of the aortic arch, passed to the right between the cervical vertebrae and the esophagus behind the right common carotid artery and internal jugular vein and then followed the normal course of the right subclavian artery.

DISCUSSION

Several papers have been written on the relations of the inferior laryngeal nerve to the inferior thyroid artery but these, in most cases, have been based on the findings in too few dissections to be of great significance and are therefore not reviewed here.

Berlin and Lahey ('29), in twenty-two dissections, found the recurrent nerve to be anterior to the inferior thyroid artery more commonly on the right side than on the left. They also mentioned the occurrence of nerves passing between branches of the artery. The data in table 2 confirm these relationships.

Ziegelman ('33) dissected forty-two cadavers and found the nerve anterior to the artery in seventeen cases, posterior in twelve, and between the branches in thirteen.

In the dissection of 400 nerves Fowler and Hanson ('29) made the following observations. "In 262 cases (65.5 per cent of 400) the nerve passed posterior to the main branches of the inferior thyroid artery; in 104 cases (26 per cent) it passed anterior; and in 34 cases (8.5 per cent) it passed between the main branches of the inferior thyroid artery." The variations in right and left sides were not separately considered. While the percentages given are not in agreement with those in table 2, the general relationship is similar: the nerve more frequently passes deep to the artery and its branches.

In the thirty-one cadavers which Nordland ('30) studied the relations were as quoted below:

Right side	*Left side*	*Both sides*
15 nerves anterior to artery (2 posterior to small branch of artery). 8 nerves posterior to artery (3 anterior to small branch of artery). 7 nerves between equal branches of artery 1 inferior thyroid artery not present.	11 nerves anterior to artery (2 posterior to small branch of artery). 11 nerves posterior to artery (1 anterior to small branch of artery). 7 nerves between equal branches of artery, 2 nerves branch around artery.	Similar in 8 cadavers; 5 had nerve anterior to artery, 1 had nerve posterior to artery, 2 had nerve between equal branches of artery.

Anomalous inferior laryngeal nerves of the same type as the three recorded here have been reported by Hooper (1887), Milianitch ('24), Work ('41) and others. Pemberton and Miller ('41) found five such anomalies and included in their paper an excellent discussion of the embryological and anatomical considerations.

SUMMARY

1. The relations of the inferior laryngeal nerves to the inferior thyroid arteries as found in 506 dissections (253 cadavers) are recorded. Twenty-eight different types of relations were found and illustrated.

2. The frequencies in which the nerve passed (a) superficial to the artery and its branches (18.6%), (b) deep to the artery and its branches (39.1%), and (c) between the branches of the artery (36.5%), are tabulated. Likewise the nerves which divided into two or more branches (5.4%) while in direct relation to the artery or its branches are recorded.

3. In three cadavers (0.6%) the right inferior laryngeal nerve was found to arise from the vagus high in the neck and therefore did not follow a recurrent course.

4. The relations of the inferior laryngeal nerve to the inferior thyroid artery were alike on the two sides in only 17% of the cadavers dissected.

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SEPARATION OF THE TWO ACIDOPHILIC GRANULOCYTES OF TURTLE BLOOD, WITH SUGGESTED PHYLOGENETIC RELATIONSHIPS

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SIX TEXT FIGURES AND ONE PLATE (TWENTY FIGURES)

INTRODUCTION

It is generally accepted at the present time that the granular leucocytes of mammals may be classified as basophiles, eosinophiles, and neutrophiles. While the first two categories present consistently a coarsely granular appearance, the third varies somewhat in the characteristics of its granules. Although in most mammals these granules are usually very small and neutrophilic, in the rabbit they are coarse and acidophilic; in the guinea pig the granules are also coarse, but amphophilic. Several other characteristics may serve to differentiate the three cell types as indicated in Jordan's monograph ('38).

Since the time that Ehrlich first proposed our present classification of leucocytes from his study of several vertebrate types, many other vertebrates have been studied so that we now are able to suggest homologous series of leucocytes in most of these animals. However, the characteristics of any one cell type may vary considerably from one class of vertebrates to another.

For example, in the birds the place of the neutrophile is taken by a granulocyte with coarse, fusiform acidophilic granules; basophiles and eosinophiles are more nearly comparable to those of mammals. In the amphibia the granulocyte types are like those of mammals. In the dipnoian fishes, as indicated by Jordan and Speidel ('31), and Champy and Louvel ('40), the granulocytes are surprisingly like those of the amphibia. The characteristics of the granulocytes in other teleost fishes are quite varied as shown by Drzewina ('11), Jordan and Speidel ('23), Robeson ('32), and Duthie ('39), but the three granulocyte types definitely are present in the group. While Drzewina ('11) and Maximow ('23) did not describe basophile granulocytes in the selachians, they did find cells with coarse, acidophilic fusiform granules, and cells with coarse, acidophilic spheroidal granules. These morphologic types would seem to correspond with similar cells of birds.

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The granulocyte relationships in the reptiles have been extremely confusing. It is the purpose of this paper to clarify the relationships of one group, the turtles. In this order (Testudinata) basophilic granulocytes have been found consistently, but the acidophilic granulocytes have presented certain difficulties. Most workers, for example, Eberhardt ('07), Werzberg ('11), Jordan and Flippin ('13), Alder and Huber ('23), and others, while describing granulocytes containing coarse, fusiform acidophilic granules as well as granulocytes with coarse, spheroidal acidophilic granules, have concluded that there is only one cytogenetic series of these cells.

On the other hand, Loewenthal ('28) and Mori ('40) apparently believe that these are two series, which might correspond with similar types in the birds. The present work indicates that, in turtles, these are actually two distinct cytogenetic series and, moreover, that they are to a certain extent physiologically comparable to the morphologically similar series in birds.

The author wishes to acknowledge his indebtedness to the Zoology Department of Duke University for supplying materials for this work, and for the kind consideration of its individual staff members. Dr. George T. Hargitt offered valuable constructive criticism. To Dr. H. E. Jordan of the Uni-

versity of Virginia the writer owes thanks for time spent in reviewing many slides, and in conference concerning the problems involved.

MATERIALS AND METHODS

Two species of turtles, from different genera of the same family, were used in the experimental work. Detailed results are given for two specimens of the box turtle, Terrapene carolina, and for three specimens of the gopher tortoise, Gopherus polyphemus. Incidental reference will be made, for comparative purposes, to the blood pictures of other vertebrate types.

Subcutaneous injections of turpentine were made into two turtles of each species. Bradley ('37) found that in chickens such injections resulted in a marked increase in the acidophiles with fusiform granules, while the numbers of the acidophiles with spheroidal granules were not specifically changed. Since nucleic acid was shown by Charipper and Davis ('32) to produce a marked change in the granulocyte relations in closely related (same family, Testudinidae) turtles (Pseudemys elegans), the third gopher tortoise was injected with nucleinic acid. The nucleinic acid (from yeast) was made in 0.7% sodium chloride solution to which was added the minimum amount of sodium bicarbonate which would allow the drug to go into solution. The amounts of the drugs and the days on which injections were made are given in the graphs (figs. 1 to 5).

Blood was drawn with a cooled syringe directly from the heart. The holes drilled through the plastrons of the box turtles, and the flaps cut in the plastrons of the gopher tortoises, were sealed with cotton and paraffin between bleedings. The total counts of erythrocytes and leucocytes were made from duplicate pipettes (1–100 dilution) with neutral red dye added to the 0.7% sodium chloride diluting solution. Permanent smear preparations were made on slides, some dried in air, some "wet-fixed" in Helly's fluid. A variety of stains was used, with the Jenner-Giemsa or Jenner-Panchrome combination (buffered to pH 6.5 for smears previously exposed for 30 seconds to osmic vapor, and to pH 6.9 for untreated, air-dried smears) being satisfactory for the air-dried smears; Maximow's azur-eosin combination was good for the wet-fixed smears (McClung, '29).

The living cells were studied in temporary preparations both stained and unstained. Dye combinations of neutral red with janus green, and of neutral red with pinacyanole, were applied after the methods of Sabin ('23) and Hetherington ('36). When the cover slips were sealed with a paraffin-vaseline mixture the preparations could be kept in an ice box [2] (6°C.) for several days before abnormalities developed. The differential counts were made from the fresh supravital preparations. The number of cells counted for each bleeding was approximately 800. The number of cells of each type per unit volume of blood was calculated from the total and differential counts. Complete determinations were made prior to the first injection. Blood-forming tissues (bone marrow and spleen) were taken for supplementary examination.

RESULTS

The box-turtle granulocyte with the fusiform or spindle-shaped granules is herein designated as the heterophile. The living cell in the fully differentiated or mature stage is replete with the specific granules. In the unstained cell the nucleus is spheroidal or, rarely, bilobed, although it may assume varying shapes during the active ameboid movement of the cell. In addition to the specific granules, a few small bodies, usually near the nucleus, may be seen with difficulty. During locomotion the cell may assume a wide variety of shapes, and under certain conditions, such as when seen in exudates, may extend 40 to 50 micra.

In the supravital preparations, with neutral red and either janus green or pinacyanole, the heterophile is easily recognized. The specific granules take up very little of the neutral red and so appear yellow to pinkish-yellow. A few neutral red bodies are present, some as small granules which do not increase in size on standing, and others as vacuoles which may increase enormously in size in the course of a few hours at room temperature. These bodies, because of their deep red color, are readily distinguishable from the specific granules. A small number of bodies staining bluish-green in either janus green or pinacyanole is always present in the form of tiny granules or slender rodlets; these are interpreted as mitochondria. Whereas the janus green may fade in a few hours, the pinacyanole is evident for many days (6°C.). These neutral red bodies and mitochondria comprise the non-specific cytoplasmic inclusions alluded to in the preceding paragraph. Occasionally there may be non-staining vacuoles of varying size, and under certain conditions refractile bodies resembling fat droplets. In many preparations the specific granules change rapidly from the typical cigar-shaped bodies to egg-shaped ones.

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[2] Dr. D. C. Hetherington suggested this procedure.

In well prepared smears, either air-dried or wet-fixed, stained with either the Romanowsky or the azur-eosin combinations, the heterophiles may be recognized clearly. The lentiform nucleus is generally peripherally located and exhibits a compact chromatin network. An indistinct halo separates the deeply basophilic peripheral portion of the nucleus from the cytoplasm containing the specific granules. The inter-granular cytoplasm is faintly acidophilic while the specific granules are deeply acidophilic (fig. 26).

During the course of the experiments after the peak of heterophilia, fewer mature stages occur in the peripheal blood while more immature stages appear. When stained supravitally the youngest heterophile myelocyte is seen to contain large numbers of cytoplasmic inclusions in the form of rod-shaped and spheroidal mitochondria, comparatively large numbers of neutral red bodies, and a few specific granules. While these specific granules may not be definitely fusiform, they are clearly oval or rodlet shaped; they are of rather uniform size in any one cell. The nucleus is rather large and vesicular, usually possessing a distinct plasmasome nucleolus. The characteristic yellowish color of the specific granules is an important distinguishing feature. In the fixed myelocytes the plasmasome nucleolus is quite evident (figs. 7, 8, 15); the cytoplasm appears as a basophilic coarse reticulum in which azurophilic granules of varying size are often present, in addition to the small number of specific granules which are to be found radiating from the centrosphere area (figs. 7 to 10, and 15 to 17).

As the cell matures the mitochondria decrease in size and numbers; the nucleus becomes more compact, with the nucleolus no longer evident; the cytoplasm becomes less basophilic while its reticular nature is gradually lost. Azurophilic granules are replaced by specific granules in increasing numbers, and which gradually acquire their mature form.

The box-turtle eosinophile with its spheroidal granules is generally somewhat smaller than the heterophile, and the two may be differentiated readily in any good preparation. This cell is less actively ameboid than the heterophile, extending usually only relatively short, blunt, pseudopods. In the supravital preparations the tiny mitochondria are often hard to locate; the neutral red bodies are few in number while large neutral red vacuoles are quite rare. The specific granules must be described as angular spheroids. They are fewer in number, less uniform in size, but appear to have greater mass, than do the heterophile granules. These granules take up the neutral red very readily and retain it without fading. The dye imparts to them a uniform copper-red color which is diagnostic for this cell in all differentiating stages.

In fixed smears it is evident that the eosinophiles are considerably more resistant to distortions due to manipulation than are the heterophiles. The granules stain a clear rose red while the intergranular cytoplasm in the region of the centrosphere is very faintly basophilic (bluish). The ovoid nucleus is to be found peripherally, is compact and deeply basophilic, but is usually not separated from the cytosome by a halo, although one is present in the cell shown in figure 25.

In one animal, Terrapene carolina 5, immature stages of the eosinophile series present at the beginning of the experiment (fig. 13) became more and more immature as the reaction to turpentine progressed (figs. 11 and 12). The earliest stages to occur in the peripheral blood possess a less compact nucleus. The specific granules are quite irregular in size and shape, but when stained with neutral red exhibit the color characteristic of the specific granules of the mature cells. Neutral red bodies are not clearly present and only relatively few small mitochondria are recorded. The cytoplasm in the fixed cells is apparently finely reticular, and uniformly basophilic, but not as deeply basophilic as that of the immature heterophiles (compare figs. 11 and 12 with 7 to 9 and 15 to 17).

In the gopher tortoise the heterophiles and eosinophiles are very similar to those of the box turtle (compare figs. 24 and 23 with 25 and 26). No complete series of eosinophiles was available for study, but the heterophile series was quite distinct. The mature eosinophile would fit nowhere into this cytogenetic series. As compared with the box turtle series, the heterophiles of the gopher tortoise possess a less coarsely reticular cytoplasm in the younger stages (compare figs. 7 and 15 with 19). The granules are less definitely fusiform in early myelocytic stages (figs. 19 to 21), but in the more mature stages (fig. 22) they are definitely fusiform.

Figure 1 shows that the reaction of the box turtle to the subcutaneous injection of turpentine was essentially a heterophilia. During the second week apparently the toxic nature of the drug was responsible for a generalized anemia and leucopenia. With the exception of the heterophiles, the only cell type which showed a consistent change was the lymphocyte. The second injection of turpentine on the seventh day apparently resulted in no specific change. During the entire experimental period the eosinophiles showed no definite change in either numbers per unit blood volume or in cytologic characteristics. The heterophiles, on the other hand, near the end of the first week contained in general more mitochondria and neutral red apparatus than at first. A tendency for the granules to be less sharply pointed was noted. On the eleventh day the few heterophiles found possessed but very

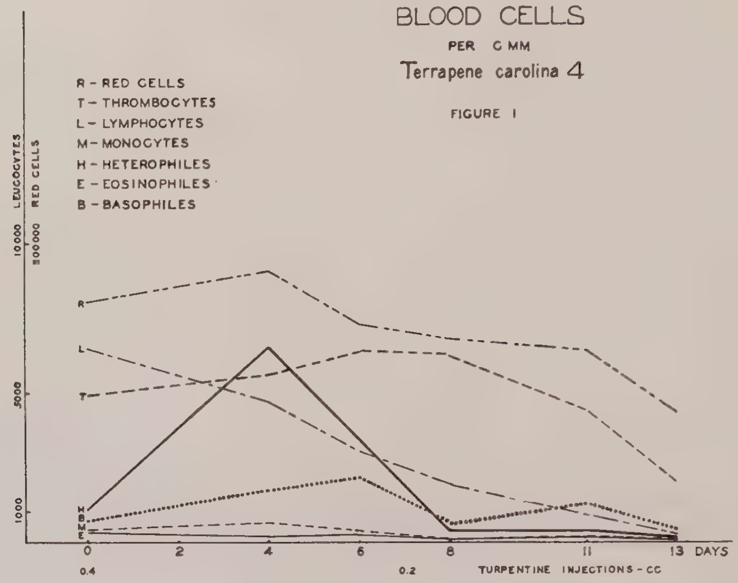
BLOOD CELLS
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FIGURE 1
R - RED CELLS
T - THROMBOCYTES
L - LYMPHOCYTES
M - MONOCYTES
H - HETEROPHILES
E - EOSINOPHILES
B - BASOPHILES
LEUCOCYTES
RED CELLS
10000
500000
5000
1000
R
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TURPENTINE INJECTIONS - CC

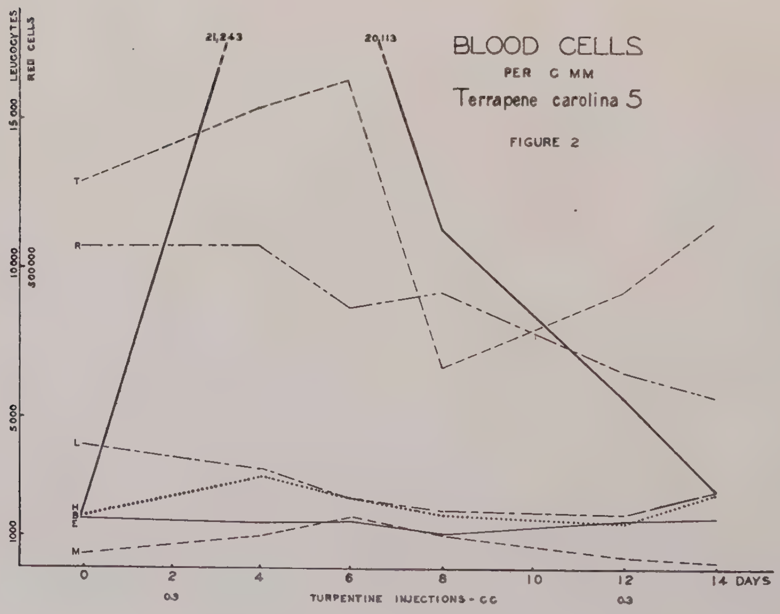
BLOOD CELLS
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FIGURE 2
LEUCOCYTES
RED CELLS
15 000
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5000
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21,243
20,113
T
R
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14 DAYS
0.3
TURPENTINE INJECTIONS - CC
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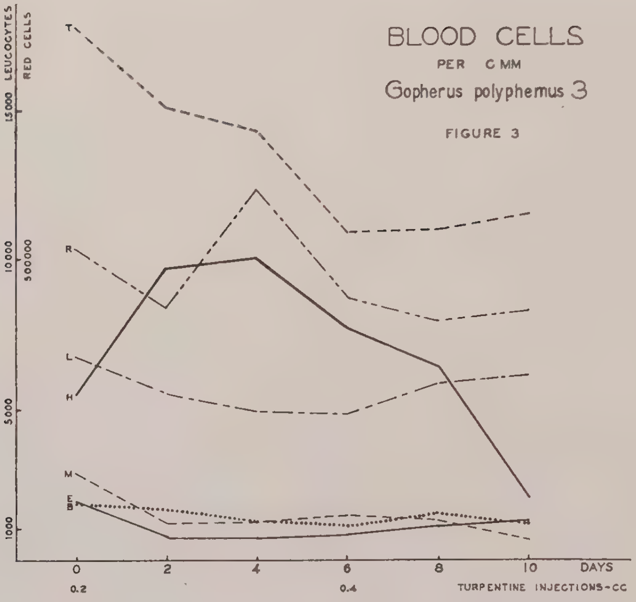
few specific granules. On the last day of the experiment (thirteenth) the cells considered to be the earliest stages of heterophiles contained large spherical bodies taking janus green, which were interpreted as mitochondria. In some of these cells there were a very few neutral red bodies along with comparatively large numbers of the mitochondria. The latter cells are definitely similar in appearance to reticular cells taken from normal bone marrow. Since a bacterial infection developed in the tissues around the opening in the plastron during the last few days, the death of the animal was possibly due to the bacteremia recorded at autopsy.

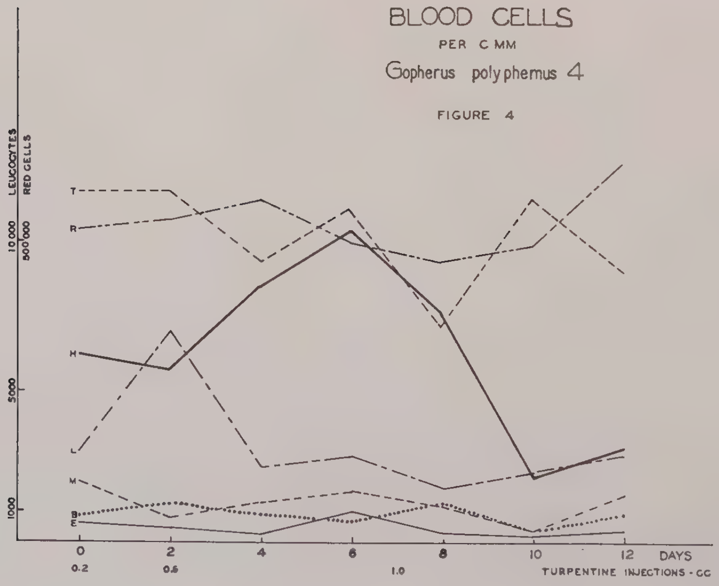
In the other box turtle (fig. 2) the reaction to turpentine seems to have been extremely exaggerated. However, since comparatively immature eosinophiles (fig. 13) were noted before injection, it may be that the animal was not normal at the beginning. Here again no definite reaction could be ascribed to the second turpentine injection. The numbers of eosinophiles per unit volume of blood remained independent of the enormous changes in the heterophile numbers. As mentioned above, the cytologic characteristics of the immature eosinophiles were quite distinct from those of the immature heterophiles. The young heterophile stages were, of course, characterized by the numerous mitochondria, which were larger and more rod-like than those of fully differentiated cells. A plasmasome nucleolus was evident in these immature heterophiles. The most immature cells present, apparently stages between reticular cells and heterophile myelocytes, had their cytoplasm thickly studded with rather large spheroidal mitochondria, which were often segregated in one-half of the cell, with the other half containing neutral red bodies alike in size. The animal was killed on the fourteenth day, with no infection recorded.

The reactions of the gopher tortoises to the injection of turpentine were essentially like those of the box turtles (figs. 3 and 4). The animals were perhaps less sensitive to the turpentine, but the procedure differentiated equally well the heterophile and eosinophile granulocytes. In both animals



BLOOD CELLS
PER C MM
Gopherus polyphemus 3

FIGURE 3

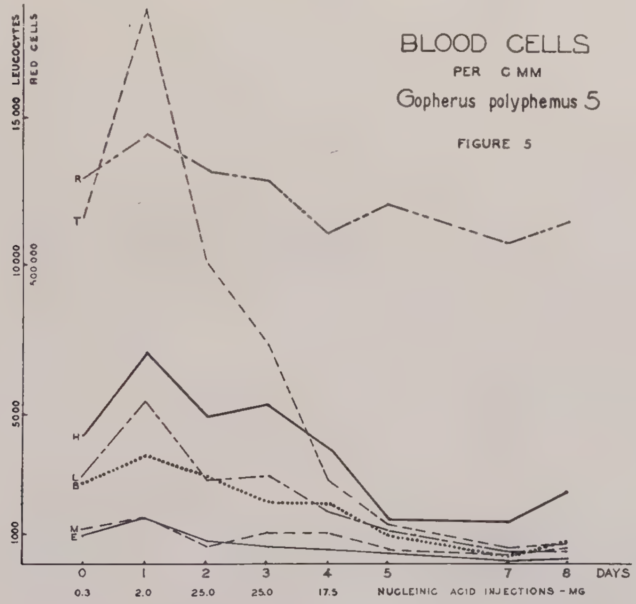


BLOOD CELLS
PER C MM
Gopherus polyphemus 4

FIGURE 4

infection in the wound was noted after the fourth day. In the last day or two of each experiment very immature heterophiles were found in the peripheral blood, along with apparently normal eosinophiles. The two cell types were distinctly and completely separable, and there was no question of the occurrence of transitional stages. The animals were sacrificed and tissues taken.

The third gopher tortoise was given nucleinic acid injections in an attempt to verify the observations of Charipper and Davis ('32), although their turtles were from a different genus (but the same family). Since it was not clear from their records the amounts of nucleic acid used,[3] rather massive injections were made. A heterophilia developed, followed by a heteropenia, (fig. 5) with the appearance of immature stages of the heterophile series (figs. 19 to 22). The numbers of eosinophiles remained relatively unaffected as compared with



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[3] Amounts should have been reported in their tables as milligrams, according to a subsequent personal communication.

other cell types. No reason was apparent for the wide fluctuation in the thrombocyte numbers of this animal.

The blood forming tissues of all animals, bone marrow and spleen, showed in general a definite depletion of blood elements. In the box turtles the marrow was extremely hypoplastic, so that the immature stages of the acidophilic granulocytes could be traced readily. These stages correspond to those found in the peripheral blood which are illustrated herein.

DISCUSSION

The study of comparative vertebrate hematology should contribute ultimately a great deal to our present knowledge of the functions of leucocytes. Comparative physiological studies are handicapped because of the lack of general agreement on the homologies of morphologic or cytologic types of cells in the several classes, and even within the same class. Until a logical set of homologies is established on a morphological basis the conclusions of physiological studies are likely to be misleading. Thus, the conclusions of Charipper and Davis ('32), based on the assumption that all acidophilic granulocytes of their experimental turtles belonged to a single cytogenetic series, could not be confirmed on the basis of the present investigation. Guieysse-Pellissier ('40) failed to consider the possibility of the presence of two types of acidophilic granulocytes in the blood of turtles he examined, even though he was concerned with the origin and fate of the alphagranules and their possible relation to pigment granules.

As indicated in the introduction, there is fundamental disagreement as to the types of granular leucocytes in the class Reptilia, and particularly in the order Chelonia. It is pertinent therefore to present the evidence accumulated to date on the characteristics of the acidophile granulocytes of turtles. With perhaps only one exception (Guieysse-Pellissier), all of the workers with the blood of turtles have recognized two morphologic types of acidophilic granules. With apparently only three exceptions they have considered these cells to belong to a single cytogenetic series. Thus Meinertz ('03), Eberhardt ('07), Hirschfeld-Kassmann ('08), Werzberg ('11), Jordan and Flippin ('13), Michels ('23), Alder and Huber ('23), Komocki ('26), Babuder ('30), Charipper and Davis ('32), Bernstein ('38), and Jordan ('38), have considered the acidophilic granulocytes as belonging to a single series, generally on the basis of their occasional observations of the so-called intermediate forms (cells containing both spheroidal and fusiform granules).

Of those mentioning two types of acidophilic granulocytes, Babuder ('30) stated that the acidophile with fusiform granules was comparable with the similar cell of birds, but he failed to separate the two acidophilic cell types in both the cell descriptions and in the tabulated differential counts. Loewenthal ('28), on the other hand, accurately described the characteristics of the two acidophilic series, but likewise failed to tabulate them differentially in his conclusions. He compared the leucocyte with fusiform granules of the turtle with that of the bird, but it is not entirely clear that he accepted the idea that there are two distinct acidophilic granulocytes in the bird. Mori ('40) has also described accurately the two acidophilic series in turtles not only from fixed smears but also from supravitally stained preparations of living cells. He mentioned another Japanese worker, Onado, who apparently considered the cell types to be separate. Mori, too, compared turtle granulocytes to similar morphologic types of bird blood.

None of these workers has clearly demonstrated the development of acidophilic granulocytes in turtles. Nor has the supposed relation between the two types been clearly described and illustrated by any worker who considered only one definitive type to be present. In reports which have included the results of differential cell counts of normal animals, and which separate the two acidophile granulocyte types, the relative percentages are found to vary greatly. Alder and Huber ('23) record 12% cells with round granules and 10% cells with fusiform granules. Loewenthal ('28) records 56.8% cells with fusiform granules, but only 5.22% cells with round granules for the same species. Mori ('40) indicates 31.4% cells with fusiform granules and 6.6% cells with spheroidal granules; these figures correspond more closely with the writer's observations on several species. Percentages of cell types are given by Michels ('23) and Charipper and Davis ('32), but are not comparable with these data. The writer has noted, as have other workers, that there is a rather consistent numerical occurrence of the two cell types in any given species. This is not consonant with the view that they represent different developmental stages of a single cytogenetic line.

When other workers have given the percentages of these cells separately they have not included a category for any intermediate or transitional type. Since most other workers have differentiated two acidophilic types, it seems safe to assume that the differential cell counts which served as the basis for the graphic results of the present work are acceptable. The fact of outstanding importance in these experimental animals is that regardless of the numbers of either cell type per unit volume of blood, or the stage of differentiation, there are no transitional stages between types.

Moreover, it is striking that, of the granulocytes, the heterophile series alone was clearly affected by the injections used (figs. 1 to 5). This of course points to a physiological difference in the reactions of the two acidophile types. Since we are interested only in the sharp separation of these two, it is not necessary to discuss changes occurring in other leucocyte types following such injections.

One of the main problems of general interest is the relation of the turtle granulocytes to those of other vertebrate orders and classes. There have been several references to the similarity in appearance between the turtle heterophile and the bird heterophile: Loewenthal ('28), Babuder ('30), Mori ('40), Wintrobe ('42), and probably others. It has been shown by Bradley ('37) that the chicken heterophile reacts specifically to turpentine injections. It is also recognized that in certain bacterial infections of chickens the heterophile reacts somewhat as does the mammalian neutrophile (Cook and Dearstyne, '34). With the reaction herein reported, showing the specific reaction of the turtle heterophile to turpentine, this cell may be homologized to a certain extent with the mammalian neutrophile.

Very little is known about the factors controlling eosinophile differentiation in birds, and obviously nothing for turtles, so that the homologies of these cells of the turtles, birds, and mammals must rest on the close similarities of cytologic properties. The eosinophiles in these animals are alike in that they contain but few, tiny, mitochondria, sparse neutral red bodies, and a trachychromatic nucleus; they lack phagocytic ability; the appearance of their specific granules is strikingly similar.

Up to the present time it has apparently not been recognized that the blood pictures of lizards and snakes are distinctly different from those of turtles. Since representatives of the Squamata have been more extensively studied than those of the Chelonia, it is only natural that homologies between the former and representatives of other vertebrate classes were attempted, rather than homologies between the turtles and other classes (except for the similarities noted by others between turtle and avian heterophiles). From a phylogenetic standpoint, however, one would expect to find closer similarities between the order Crocodilia and the birds than with any of the other three reptilian orders. An examination of the blood of several alligators indicates to the writer that the acidophilic granulocytes are heterophiles and eosinophiles as in turtles. Komocki ('36) reports that there are two morphologic types (fusiform, and spheroid) of acidophilic granulocytes in the Rhynchocephalia (Sphenodon). With the exception of one report, Pena Roche ('39), no observer has seen two similar types of granulocytes in the Squamata. This author, however, gives apparently accurate descriptions of two types of acidophilic granulocytes in two species of the South American lizard Liolaemus.

The homologies of Sauropsidan heterophiles and eosinophiles are shown in figure 6, the examples being taken from the writer's slides. Although some earlier workers, such as Dantschakoff ('09); Kollmann ('12); and later Lundquist and Hedlund ('25); and Loewenthal ('30), considered the avian heterophiles and eosinophiles to be merely different phases of a single cell type, many other workers have described these as two distinct series. Today the opinion seems to be that these are undoubtedly two distinct cell types; Forkner ('29); Shaw ('33); Cook and Dearstyne ('34); DeVilliers ('38); Bradley ('37); Twisselmann ('39); and Wintrobe ('42).[4]

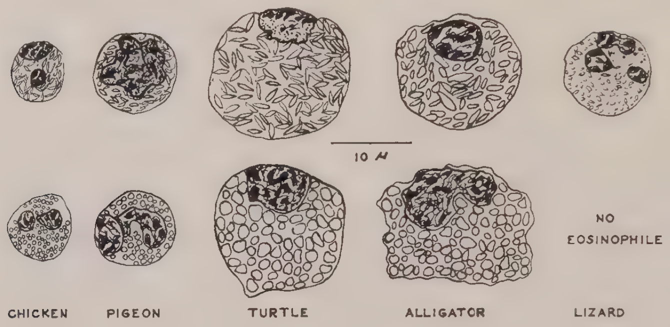

FIGURE 6 SAUROPSIDAN HETEROPHILES & EOSINOPHILES

In some turtles the morphological characteristics of the granules are not as clear-cut as in the species used in this study. This is probably responsible in part for the failure of other workers to recognize two distinct series. The tendency of the fusiform granules to round up under certain conditions may also be partly responsible, as well as the fact that the less mature heterophiles possess less sharply fusiform granules. In denying the existence of two acidophile types in chickens Lundquist and Hedlund ('25) pointed out that the osmotic pressure of the medium could be responsible for changes in the shape of the fusiform granules.

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[4] Forkner ('29) and Wintrobe ('42) call the cell with fusiform granules the eosinophile, and the cell with spheroidal granules the pseudo-eosinophile. This does not now seem logical considering the cytological and physiological characteristics of this cell with fusiform granules, as well as the fact that it occurs in most birds in greater numbers than does the type with spheroidal granules. In some birds, it is indicated by Cullen ('03) and by Magath and Higgins ('34), the acidophile with spheroidal granules occurs in greater numbers than does the cell with fusiform granules. However, Mori ('40), working with ducks as did Magath and Higgins (Anas platyrhyncha domestica and Anas platyrhynchos platyrhynchos respectively), found the cells with fusiform granules more abundant.

In the common mud turtle, Kinosternon subrubrum, (Family Kinosternidae), the shapes of the eosinophile granules are so irregular that they may be confused with the heterophile granules, although the staining reactions and other differential characteristics may be safely used to separate the granulocyte types. Mori ('40) noted that while in one species (Clemmys japonica) the eosinophile granules were round, in another species (Geoclemmys reevesii) they were oval.

The differentiation of the two acidophile granulocytes of alligator blood is based on examinations of supravitally stained cells and of fixed smear preparations only. In the living cells the heterophile granules are not spindle shaped, but are cylindrical rodlets of varying lengths, much like small twigs. The eosinophile granules are like those of most turtles. In the fixed smears the granules are not so dimorphic; the extremely small numbers of eosinophiles in the blood of the animals examined made it difficult to classify these cells accurately.

Not only are the cytological characteristics of the Sauropsidan heterophiles (and of the eosinophiles), very similar but, as above indicated, the physiological characteristics of the heterophile series are also similar.

This line of reasoning leads to the problem of the classification of another reptilian leucocyte type which generally has been classified as a neutrophile (Loewenthal, '28; Babuder, '30; Jordan, '38; etc.). This problem cannot be fully discussed at this time, but there are several observations which indicate that this cell is more closely related to the monocytes of other classes than it is to the finely granular leucocytes (neutrophiles or heterophiles). These may be enumerated as follows:

1. The disagreement of workers over its classification indicates that its granulocyte characteristics are not clear cut.
2. A typical granulocyte cell lineage has never been demonstrated.
3. The fact that in its most fully differentiated stage it still possesses a comparatively large amount of mitochondrial substance does not correspond with the small amounts of mitochondrial substance found in typical, mature, granulocytes.
4. It always possesses, in its most fully differentiated stage, a comparatively large and active neutral red apparatus. This is probably associated with its phagocytic ability, which is developed beyond that of typical granulocytes.
5. It fails to react specifically to injections of turpentine, thyroxine, and nucleinic acid, as do the heterophiles of other vertebrates.

Werzberg ('11) and Mori ('40) classified these cells as non-granular (poly- or ampho-chromatophile) leucocytes.

The granular leucocytes of amphibians are remarkably like those of most mammals, even though some are much larger cells. The blood cells of dipnoian fishes (Jordan and Speidel, '31; Champy and Louvel, '40) are very large, with the leucocytes quite similar to those of some amphibians, although the granules of the dipnoian neutrophile are much more distinct.

The conditions in the other teleost fishes cannot be discussed briefly because of the wide diversity of types and the relatively small amount of work that has been done with them. It is interesting to note that Duthie's observations ('39) on the transformation of a basophilic granulocyte into an acidophilic one as the cell migrates to epithelial surfaces (in the Family Triglidae), is strongly suggestive of the descriptions of Jordan and Speidel ('31). It may also be useful in studying the reasons for the apparent lack of the eosinophilic series in the majority of the Squamata.

In the more primitive group of fish, the selachians, the granulocytes are undoubtedly more similar in appearance to those of Sauropsida than are those of any other group. Even here the interrelations of granulocytes are not entirely clear since Maximow ('23) indicated that the cell with fusiform granules in two species should be called the heterophile (Spezialleukozyte), while the cell with fusiform granules of a third species classified as the eosinophile. This anomalous condition was described apparently only on the basis of staining reactions, and should be studied further on the basis of physiological reactions.

It is necessary to mention the work of Reznikoff and Reznikoff ('34) in this connection. These workers injected turpentine intraperitoneally into sharks (Mustelis canis). They assumed that fish lack leucocytes homologous with the polymorphonuclear leucocytes of mammals, so were endeavoring to determine what cell type apparently replaced them. They concluded that lymphocyte production was stimulated by turpentine, and therefore this cell had the same significance as the mammalian neutrophile. The tabulated results were dependent upon total and differential cell counts. The characteristics of the various leucocyte types were not mentioned, either from description and illustrations or from reference to previous work. It may well be that the varying reactions in other cell types indicated, but not discussed, are also significant in this experiment.

The characteristics of the granulocytes of the cyclostomes, as described by Jordan and Speidel ('30), are apparently more like those of the amphibians than like those of any other group.

Throughout this discussion an attempt has been made to correlate the present knowledge concerning the acidophilic granulocytes of vertebrates so that the phylogenetic relationships of these cells can be investigated from a broader standpoint. A detailed consideration of the cytological characteristics of these cells in the various vertebrate groups has not been given. Such a consideration will be of greater value after the physiological characteristics of these cells have been more thoroughly studied.

SUMMARY AND CONCLUSIONS

In turtles there are two distinct coarsely granular acidophilic leucocytes which, in the mature stages, are distinguished by the fusiform or spindle shaped granules of the heterophile, and the spheroidal granules of the eosinophile.

Under experimental conditions, developmental stages of the heterophile series are present in the peripheral blood side by side with normal mature eosinophiles. The two series are separable in all developmental stages, both in supravital and in fixed preparations. The earlier stages of each are recognizable in hypoplastic hematopoietic tissues. There are no transitional stages between the two cytogenetic series.

The heterophile and eosinophile are physiologically distinct since the former reacts to injections of turpentine and nucleinic acid, while the latter apparently does not.

The acidophilic granulocytes of turtles are very similar to those of birds.

With respect to acidophilic granulocyte homologies, the vertebrates discussed may be grouped into two lines: one line contains the selachians, reptiles, and birds; the other contains the cyclostomes, teleosts, amphibians, and mammals.

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PLATE

PLATE 1[5]

EXPLANATION OF FIGURES

Acidophilic granulocytes of two species of turtles. Table level camera lucida drawings from blood smears. × 900.

T.c., Terrapene carolina; J-P, Jenner-Panchrome; d.s., air-dried smear; G. p., Gopherus polyphemus; J-G, Jenner-Giemsa; w. s., wet-fixed smear.

7 Heterophile myelocyte, T. c. 5, J-P stain, d. s.
8 Heterophile myelocyte, T. c. 5, J-P stain, d. s.
9 Heterophile myelocyte, T. c. 5, J-P stain, d. s.
10 Heterophile myelocyte, T. c. 5, J-P stain, d. s.
11 Eosinophile myelocyte, T. c. 5, J-P stain, d. s.
12 Eosinophile myelocyte, T. c. 5, J-P stain, d. s.
13 Eosinophile myelocyte, T. c. 5, J-P stain, d. s.
14 Eosinophile myelocyte, T. c. 5, J-P stain, d. s.
15 Heterophile myelocyte, T. c. 4, J-P stain, d. s.
16 Heterophile myelocyte, T. c. 4, J-P stain, d. s.
17 Heterophile myelocyte, T. c. 4, J-P stain, d. s.
18 Heterophile myelocyte, T. c. 4, azur-eosin stain, w. s.
19 Heterophile myelocyte, G. p. 5, J-G stain, d. s.
20 Heterophile myelocyte, G. p. 5, J-G stain, d. s.
21 Heterophile myelocyte, G. p. 5, J-P stain, d. s.
22 Heterophile myelocyte, G. p. 5, J-P stain, d. s.
23 Mature heterophile, G. p. 5, J-P stain, d. s.
24 Mature heterophile, G. p. 5, J-P stain, d. s.
25 Mature eosinophile, T. c. 4, Wright's stain, d. s.
26 Mature heterophile, T. c. 4, Wright's stain, d. s.

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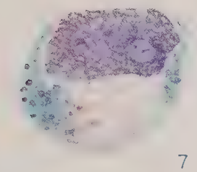
[5] Reproduction in color was made possible through the generous support of the Charles W. Hargitt Fellowship Committee.

ACIDOPHILIC GRANULOCYTES OF TURTLES
DWIGHT L. RYERSON

PLATE 1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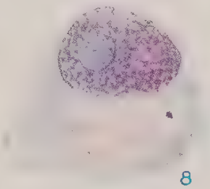
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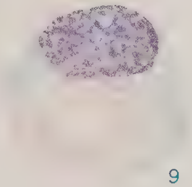
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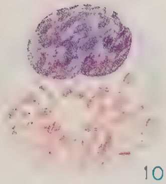
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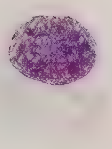
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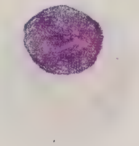
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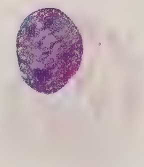
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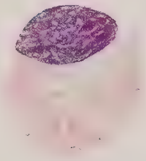
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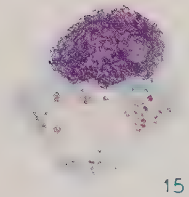
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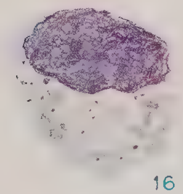
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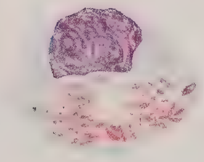
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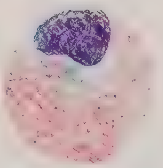
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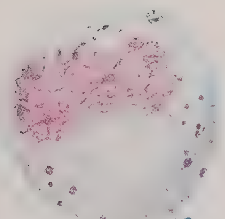
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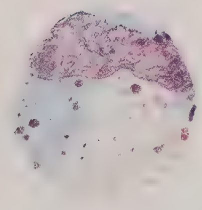
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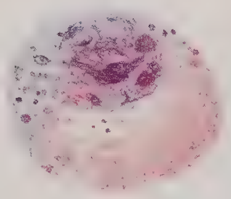
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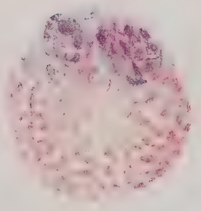
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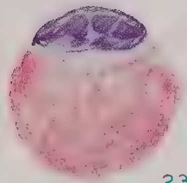
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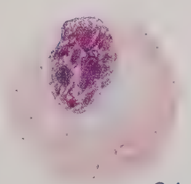
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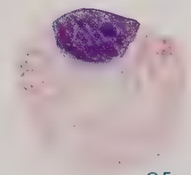
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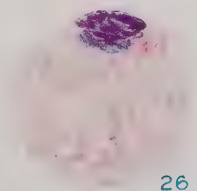
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CHANGES IN THE BIREFRINGENT MATERIAL IN THE ADRENAL CORTEX OF THE RAT FOLLOWING ADMINISTRATION OF ADRENOTROPHIC HORMONE[1]

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ONE PLATE (FOUR FIGURES)

The importance of the trophic effect exerted by the anterior pituitary on the adrenal cortex of the rat has been known since the work of Smith ('30) who called attention to the cortical atrophy, the diminution in cytoplasmic content of the cortical cells, and the pronounced loss of cortical lipids which follows hypophysectomy. Smith noted, further, that this deranged morphology could be restored to normal by the use of transplants of anterior pituitary tissue.

In 1933, Collip, Anderson and Thomson succeeded in preparing extracts of the anterior pituitary which acted selectively on the adrenal cortex. It was shown that injections of this substance, the adrenotrophic factor, not only produced hypertrophy of the adrenal cortex in the intact rat, but that it also effectively repaired the damaged adrenal cortex in hypophysectomized animals.

Emery and Atwell ('33) confirmed the stimulating effect of this anterior pituitary principle on the adrenal cortex, and claimed that the response was more marked when it was injected into castrated animals. Their experiments indicated that in animals injected with adrenotrophe the cells of the

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[1] This investigation was aided by a grant from the Committee on Research in Endocrinology of the National Research Council.

fasciculate and reticulate zones of the adrenal cortex were enlarged and contained more lipid than in normal animals. Emery ('33) commented that the dosage of pituitary transplants or of a crude extract of anterior pituitaries which is required for adrenal growth is many times the amount required for noticeable enlargement of the ovaries.

Evans, Pencharz, Meyer and Simpson ('33) reported that the administration of extracts of the anterior pituitary, rich in growth hormone, to hypophysectomized rats stimulated the adrenals sufficiently to maintain the pre-operative weights of these glands. Microscopic studies of the stimulated adrenals showed them to be of reasonably normal morphology except that the lipid content of the cells remained below normal. Injections of prolan or of any preparation made from pregnant mare serum into hypophysectomized rats failed to maintain the weight of the adrenals at their pre-operative levels. No histological evidence indicating response of these adrenals was found when gonad-stimulating hormone was administered although such treatment in adult females produced ovaries weighing 1000 mg. and effected repair of fertility in males.

Houssay, Biasotti, Mazzocco and Sammartino ('33) demonstrated that administration of alkaline extracts of the anterior pituitary to hypophysectomized dogs was followed by hypertrophy of the adrenals and an increase in the lipid content of the cortical cells. Schaefer ('33) performed subcutaneous implantations of a single pituitary every other day for a period of 30 days in hypophysectomized garter snakes. Although this treatment did not restore the adrenals to normal size, it was noted that the cortical cells regained approximately their normal nuclear and cytoplasmic appearance. Emery and Winter ('34) claimed that following complete thyroidectomy, the usual hypertrophy of the adrenal brought about by anterior pituitary extracts did not occur, an observation which has been confirmed by other workers. These investigators reported that castration per se had no effect on the adrenal.

Reiss, Bálint, Oestreicher and V. Aronson ('36) reported that the lipid in the adrenal cortex of a normal rat is in the form of relatively fine granules spread diffusely over the entire cortex. In the hypophysectomized rat, on the other hand, these granules are coarser and appear to be clumped. This latter condition suggested to them that the coarse, clumped granules of cortical lipid are not involved in the production of adrenal cortical hormone, but rather that they indicate storage of the hormone. These investigators also demonstrated that adrenotrophic hormone reduced the enlarged "sudanophobic zone," which is said to be characteristic of the adrenal cortex in hypophysectomized rats, to normal width.

Moon ('37) called attention to this observation of Reiss, Bálint, Oestreicher and V. Aronson ('36) on the occurrence in normal adrenals of an intermediate zone between the glomerulosa and fasciculata in which there is little or no lipid. Moon reported that the adrenals of intact rats treated with adrenotrophic hormone showed hypertrophy and hyperplasia of the cortical cells with the hyperplasia being limited to the cells of the glomerulosa and the outer fasciculata. An increased lipid content was found throughout the entire cortex of the treated animals, with one of the earliest signs of stimulation being the presence of lipid within the cells of the normal lipid-free zone of the cortex.

Davidson ('37) produced a marked hypertrophy of the adrenals in castrated and in castrated-hypophysectomized rats by administering 250 to 450 units of adrenotrophic hormone over a period of 9 to 14 days. No evidence of cortical hyperplasia was found, and the adrenal hypertrophy was thought to be due solely to cellular enlargement.

Noble and Collip ('41) published the results of an interesting series of experiments in which the activity of adrenotrophic hormone was augmented by simultaneous injections of weak saline suspensions of whole ox pituitary. This treatment produced the greatest adrenal hypertrophy so far recorded. Concomittant with the adrenal hypertrophy, they noted an enlargement of the preputial glands, but not of the seminal vesicles or prostate. It was suggested that either the augmented adrenotrophe caused the liberation of a different substance by the adrenals than did administration of adrenotrophic hormone alone, or that more than one type of adrenotrophe exists.

Golla and Reiss ('41–'42) reported the presence of two physiologically different adrenotrophic hormones. According to them "Adrenotrophe-A," is an adrenal cortical-stimulating hormone which may be extracted from the anterior pituitary. Injections of this substance into hypophysectomized rats will check the further atrophy of the adrenals or will lead to an increase in adrenal weight. According to these investigators this is the only adrenotrophe which will cause disappearance of the "sudanophobic zone." On the other hand, injections of "Adrenotrophe-B," which is found in pregnant mares' serum, into hypophysectomized rats will check the further atrophy of the adrenals or will lead to an increase in adrenal weight, but will not affect the "sudanophobic zone" of the cortex. "Adrenotrophe-B" will withstand boiling, an indication that it bears no relation to the gonadotrophic factors. It should be noted that Evans, Pencharz, Meyer and Simpson ('33) were unable to demonstrate any adrenotrophic effect following administration of serum from pregnant mares.

EXPERIMENTAL PROCEDURE AND RESULTS

In the present study albino rats of both sexes were castrated at 28 days of age. Beginning 5 days later the experimental groups received subcutaneous injections of adrenotrophic hormone [2] morning and evening for 20 consecutive days. One group received a total of 200 mg. of the powdered hormone, another group received 400 mg., a third group received 500 mg., while a fourth group received 800 mg. (table 1). In each case

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[2] The preparations of adrenotrophic hormone used in this study were supplied through the kindness of Dr. Oliver Kamm of Parke, Davis & Co. The assay values for adrenotrophic activity on the basis of amounts required to double the weight of adrenals in hypophysectomized immature rats were 20 mg. for 1034 B and 35 mg. for 1056 B. The values for lactogenic hormone, which is commonly found in association with the adrenotrophic factor, were 0.67 pigeon units per milligram for 1034 B and 5 units per milligram for 1056 B. From these figures it is apparent that 1034 B represents a much better preparation of adrenotrophe than 1056 B.

TABLE 1

GROUP I Adrenotrophe No. 1034 B

Description of animals	Sex	Number of animals	Body weight (grams)				Actual thymus weight (mg.)		Actual adrenal weight (mg.)		Thymus weight (mg.) per 100 grams of body weight		Adrenal weight (mg.) per 100 grams of body weight	
			At castration		At sacrifice									
			Average	Extremes	*Average*	Extremes	*Average*	Extremes	*Average*	Extremes	*Average*	Extremes	*Average*	Extremes
Normal controls	♂	2	*73*	70-76	*147*	136-158	*253*	250-256	*39*	36-42	*173*	158-188	*27*	27-27
	♀	2	*68*	66-70	*131*	124-138	*257*	208-306	*42*	40-44	*199*	151-247	*32*	32-32
Castrate controls	♂	6	*65*	60-76	*134*	115-156	*390*	230-450	*36*	32-41	*290*	200-381	*27*	26 28
	♀	5	*64*	60-74	*128*	110-152	*416*	278-503	*38*	28-46	*324*	253-390	*30*	26-33
Castrate, 200 mg. adrenotrophe	♂	2	*63*	58-68	*136*	132-140	*160*	157-164	*53*	46-57	*118*	112-124	*38*	35-41
	♀	1	74		126		101		65		87		52	
Castrate, 400 mg. adrenotrophe	♂	3	*72*	66-78	*128*	115-140	*110*	63-159	*71*	58-83	*88*	49-138	*56*	51-59
	♀	4	*66*	60-72	*116*	102-130	*97*	39-141	*64*	53-70	*82*	38-109	*56*	41-59
Castrate, 500 mg. adrenotrophe	♂	3	*58*	52-66	*102*	96-112	*31*	10-46	*71*	69-74	*30*	11-41	*69*	63-74
	♀	2	*52*	46-58	*86*	76-96	*27*	11-42	*78*	63-93	*28*	14-43	*94*	66-122

GROUP II Adrenotrophe No. 1056 B

Description of animals	Sex	Number of animals	Body weight (grams)				Actual thymus weight (mg.)		Actual adrenal weight (mg.)		Thymus weight (mg.) per 100 grams of body weight		Adrenal weight (mg.) per 100 grams of body weight	
			At castration		At sacrifice									
			Average	Extremes	*Average*	Extremes	*Average*	Extremes	*Average*	Extremes	*Average*	Extremes	*Average*	Extremes
Normal controls	♂	1	72		138		380		28		275		20	
	♀	2	*66*	64-68	*136*	133-139	*352*	345-359	*36*	31-40	*266*	262-269	*26*	23-29
Castrate controls	♂	2	*77*	70-85	*167*	156-178	*412*	317-507	*39*	35-43	*252*	178-325	*23*	22-24
	♀	2	*85*	79-90	*153*	138-168	*489*	324-645	*37*	33-41	*310*	235-385	*24*	24-24
Castrate, 800 mg. adrenotrophe	♂	1	90		136		74		53		54		39	
	♀	2	*97*	96-98	*147*	139-154	*129*	103-155	*64*	55-72	*87*	74-101	*43*	40-47

the animals were sacrificed at 53 days of age. Groups of normal and experimental controls were carried through the same schedule.

The powdered adrenotrophic hormone was dissolved in a buffered solution consisting of 1000 cc. distilled water, 200 mg. tri-sodium phosphate and 5 cc. 95% phenol. After the adrenotrophic hormone was completely dissolved the mixture was neutralized with normal phosphoric acid and the solutions adjusted so that each animal received its daily allotment of hormone in 2 or 3 cc., the larger amount being used in the group which received 800 mg. of adrenotrophe.

The adrenal glands were removed at autopsy, weighed, fixed in 10% neutral formalin and embedded in gelatin after the method of Zwemer ('33). Each gland was subsequently sectioned at 10 microns by the frozen-section method, and every fifth section was studied, unstained, with the polarized-light microscope.

The polarized-light or petrographic microscope differs from the ordinary instrument in that a polarizer is placed below and an analyzer above the tissue which is being examined. The polarizer and analyzer consist of Ahrein's prisms crossed in such a manner that polarized light emitted by the polarizer is refracted away from the eye of the observer by the analyzer. If the tissue under examination is optically inactive no light reaches the eye and the tissue appears dark gray or black. On the other hand, if the tissue is optically active, i.e., if it is capable of bending the rays of polarized light emitted by the polarizer sufficiently to pass through the analyzer, the tissue can be observed through the microscope. Tissue sufficiently optically active to be observed through this specialized microscope is termed birefringent. Birefringent materials, when studied with polarized light show in one complete rotation of the stage of the microscope four points of illumination and four points of extinction, and may or may not show the Maltese cross of polarization. Most tissues are optically active in part, so that in polarized light the optically active (birefringent) structures are highly illuminated as compared with the dark background formed by the optically inactive parts of the tissue.

Our observations have led us to believe that the quantitative and qualitative distribution of the birefringent material of the adrenal cortex of the rat is closely associated with the production of adrenal cortical hormone.[3] We believe, further, that at least some of this material is discharged into the capillaries in the form of, or closely associated with, small intra-cytoplasmic dust-like particles of birefringent material. In a resting gland these small dust-like birefringent particles may be seen readily within the cytoplasm of the cortical cells. It is believed, further, that the hormone is stored in, or closely associated with, larger intra-cytoplasmic globules of birefringent material. This supposition would be in agreement with Bennett's ('40) observation that crystals of desoxycorticosterone acetate are birefringent when examined with polarized light.

The adrenal cortex of a normal animal as seen through the polarized-light microscope (fig. 1) may be divided into four zones. The *outer zone,* comprising the zona glomerulosa, is relatively poor in birefringent material. The optically active material is usually present in the form of medium-sized cytoplasmic globules. Small dust-like particles of birefringent material may or may not be present. The *optically inactive zone,* corresponds to the "sudanophobic zone" of Reiss and others ('36). It is a relatively narrow zone located between the glomerulosa and outer fasciculata, and its cells are usually devoid of birefringent material. The *optically active zone,* consists of all or most of the zona fasciculata. In general, this zone corresponds with the "secretary zone" of Bennett ('40). Its appearance under polarized light is subject to considerable variation. Usually its cells are well filled with small dust-like particles of birefringent material. Larger globules of all sizes and of marked birefringency are commonly seen in many of the cells. These large, irregularly-shaped birefringent globules are responsible for the relatively large amount of polarized light transmitted by this zone. The *inner zone* usually comprises the inner one-third or more of the cortex. This zone is commonly relatively poor in birefringent material, which may be in the form of small dust-like particles and/or the larger, irregularly-shaped globules.

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[3] Our use here of the term "adrenal cortical hormone" is not restricted to the physiologically active substances which are ordinarily regarded as cortical hormones, i.e., those factors which are concerned with salt, water and carbohydrate metabolism, since it is now apparent that the adrenal cortex produces a wide variety of hormones (Wintersteiner, '42). These include androgenic and estrogenic substances as well as the hormones which are usually regarded as more typically identified with the adrenal cortex.

The adrenal cortices of fifteen untreated castrate rats (eight males and seven females) appeared somewhat different from the cortices of normal controls (fig. 2). As a rule there is some loss of the small dust-like particles of birefringent material, especially in the optically active zone. In some cases the dust-like particles were markedly deficient throughout the cortex. The large, irregularly-shaped globules of birefringent material are usually present in greater numbers, especially in the inner zone. In approximately one-half the cases, the optically inactive zone is obscure or absent. In these castrated animals no differences were observed in the adrenals of the two sexes. Since the nature of the birefringent material in the adrenals of these castrated rats is indicative of a depletion of cortical hormone, it is worth noting that the adrenals are regarded as a source of sex hormones found after castration in men and animals (Wintersteiner, '42). The nature and distribution of the birefringent material in the cortices of our castrated rats suggests the possibility that an attempt was being made to compensate for the loss of sex hormones which are normally produced by the gonads.

The adrenal cortices of the castrated animals which had received either 200 mg. (two males and one female) or 400 mg. (three males and four females) of adrenotrophic hormone (1034 B) over a period of 20 days (fig. 3) were characterized by an hypertrophy of approximately 70%. The dust-like particles of birefringent material were reduced greatly or absent entirely, and the cortex could no longer be divided into four zones on the basis of qualitative and quantitative differences in the birefringent material. The optically active material was almost entirely in the form of large, irregularly shaped globules scattered more or less evenly throughout the cortex. In this series a common observation was the presence of small dust-like particles of birefringent material within the walls or lumina of the capillaries. This is in accord with the observations of Deanesley ('31), Singer and Zwemer ('34) and of Zwemer ('36) who interpreted the presence of small fat-bodies in the adrenal capillaries as evidence that they had been secreted by the cortical cells. The cortical hypertrophy observed in the animals of this series, together with the presence of dust-like particles of birefringent material in the cortical capillaries and the marked depletion of this material within the cortical cells, has led us to believe that these adrenal cortices were releasing hormone about as rapidly as it was formed.

The adrenals of the castrated animals which had received 500 mg. of adrenotrophic hormone (1034 B) over a 20-day period (three males and two females) showed an hypertrophy of approximately 100% (fig. 4). The adrenal cortices of these animals, as studied by polarized light, differ markedly from those of preceding groups. For purposes of description, the cortex may be divided into three areas, namely, an outer one-fourth, a middle one-half and an inner one-fourth. The outer one-fourth appears to be formed by a fusion of the outer and the optically inactive zones. The cells are packed with large, irregularly-shaped globules of highest birefringency. These globules are responsible for the transmission of the large amount of polarized light in this area. The middle one-half of the cortex, consisting of the zona fasciculata, is characterized by a rather heavy deposition of small and medium-sized intra-cytoplasmic globules of relatively low birefringency superimposed on a fairly heavy background of small dust-like particles of birefringent material. The inner one-fourth of the cortex, comprising the zona reticularis, differs in appearance from that of the middle two-fourths in that it also contains some large, highly birefringent, intra-cytoplasmic globules. Occasionally these globules were so large as to fill an entire cell. Since a nucleus could not be observed in these cells they may be regarded as dead.

A last group of castrated animals (one male and two females) were injected with 800 mg. of adrenotrophic hormone (1056 B) over a period of 20 days. Hypertrophy of the adrenals, as well as the microscopic appearance of the adrenal cortices, were identical with those described for the animals which had received either 200 or 400 mg. of adrenotrophe 1034 B. The adrenotrophe injected into this particular group of animals was a different preparation than had been used in the other groups. Assays of this preparation (adrenotrophe 1056 B) have shown that it contained appreciably less adrenotrophic hormone than the extract (adrenotrophe 1034 B) administered to the first three experimental groups reported in this paper. Although adrenotrophe 1056 B was relatively poor in adrentotrophic hormone, it was found to contain appreciable quantities of lactogenic hormone.[2]

DISCUSSION

According to present-day concepts, adrenal cortical cells arise from undifferentiated cells of the capsule and pass centripetally to expire near the cortico-medullary border. During their progress inward from the capsule the cortical cells gradually accumulate lipid, the lipid-content of these cells reaching its maximum while they are within the zona fasciculata. In the adrenals of our normal animals the presence of birefringent material within the cells of the outer zone, its absence in the cells of the optically inactive zone and its reappearance within the cells of the optically active zone is difficult to reconcile with the commonly accepted theory regarding the maturation of spongiocytes. It would appear to us quite logical to assume that each cortical cell may produce and discharge birefringent material during at least two different stages in its life cycle — once while it is within the zona glomerulosa and at least once more while it is within the fasciculata. It would be useless at this time to speculate as to whether these different stages are involved in the production of separate hormones.

It would appear from these studies that the administration of considerable quantities of adrenotrophic hormone to castrated rats stimulates hypertrophy of the adrenals. The hypertrophy appears to be limited to the cortex, and the extent of this process bears a direct relationship to the potency of the adrenotrophe which is administered.

A considerable re-arrangement of birefringent material occurred in the adrenal cortex when the quantity of adrenotrophe administered over a period of 20 days was between 200 and 400 mg. In the adrenal cortices of rats so treated the small dust-like intra-cytoplasmic particles of birefringent material were much depleted or absent. The presence of these dust-like particles within the walls or lumina of the capillaries is interpreted as an indication that adrenal cortical hormone [3] was being secreted actively and released rapidly by these glands. The nature of this hormone remains open to question. As noted earlier in this paper it is believed that the adrenal cortex produces a variety of physiologically active substances. These include at least two hormones or groups of hormones which are commonly identified with cortical function, viz., those with an atom of oxygen on C_{11}, related to glyconeogenesis and muscle efficiency and those without an atom of oxygen on C_{11}, involved in the control of salt and water metabolism. Grattan and Jensen ('40) have shown that the adrenotrophic factor will stimulate production of the first group of hormones. There is no satisfactory evidence that the adrenotrophic hormone influences the production of the second group.

In addition to these substances the cortex secretes various sex hormones. Our own evidence for enhanced activity of the adrenals in the animals which received adrenotrophic hormone bears upon the production of sex hormone only. As has been noted in an earlier publication (Nelson, '41), the sex accessory organs of these castrated animals which received adrenotrophic hormone show evidence of stimulation by sex hormones. This was true in both males and females and can be explained most logically on the basis of the production of sex hormones in the animals' own adrenals. The evidence gained from a study of the thymus glands of our animals (table 1) shows only the presence of some substance which induces an involution of the thymus that is proportional to the hypertrophy of the adrenal cortex. This may be either one of the sex hormones or one of the substances commonly identified with cortical function, particularly one having an atom of oxygen on C_{11}, as shown by Korenchevsky, Dennison and Simpson ('35) and Ingle ('38).

When still greater quantities of adrenotrophe were administered, i.e., 500 mg. over a period of 20 days, the adrenal cortex was characterized by a marked storage of birefringent material. Most of the optically active material was in the form of large, highly birefringent, intra-cytoplasmic globules. No other experimental procedure conducted by us has produced adrenals in which this storage phenomenon is so marked. The appearance of the glands of this group has led us to believe that secretion is proceeding so rapidly that complete release of hormone is not accomplished. Under these circumstances an accumulation of secretory products would be expected.

The appearance of the adrenal cortical cells in the outer and inner fourths of the cortices in animals which had received 500 mg. of adrenotrophic hormone over a period of 20 days are particularly interesting. According to Zwemer ('36), the glomerular cells arise from indifferent connective tissue-like cells in the capsule. They soon lose their long processes, assume an ovoid shape and lipid droplets appear in the cytoplasm. A further increase in the amount of cytoplasm and a further increase in cell lipids mark the transition to spongiocytes. However, the present studies show that the administration of large quantities of adrenotrophic hormone produces glomerular cells which contain a much greater quantity of birefringent material than is seen in the spongiocytes.

According to Zwemer ('36), one of the characteristics of the so-called lipid storage type of gland is the reduction of the reticularis to a small band of cell cords. As indicated by the present studies, administration of large quantities of adrenotrophic hormone increases the quantity of the birefringent material in the zona reticularis. The cells of this zone contain more birefringent material than would be seen normally in the cells of the glomerulosa although less than in those of the fasciculata.

The microscopic picture of the adrenal cortices of the rats which had received 200 to 400 mg. of adrenotrophic hormone appears to be similar to that seen in the adrenals of animals subjected to the influence of certain toxins (Deanesly, '31; Hoerr, '31; and others). That the injected solutions are not in themselves toxic is indicated by the marked storage of birefringent material in the adrenal cortices of rats administered still greater quantities of adrenotrophic hormone. To our knowledge, such a marked storage of birefringent material in the adrenal cortex has not been produced by any experimental means other than by the administration of adrenotrophic hormone.

SUMMARY

The adrenal cortex of normal rats, as seen through the petrographic microscope, is described. On the basis of the quantitative and qualitative differences in birefringent material, the normal adrenal cortex has been divided into four zones. The slight differences in the appearance of the adrenal cortex in normal and in castrated rats are described.

The adrenal cortices of castrated animals administered 200 to 400 mg. of adrenotrophe over a period of 20 days were hypertrophied and showed a marked change in the distribution and character of the optically active material, particularly in the direction of a depletion of the small dust-like particles. The presence of these dust-like particles of birefringent material within the walls and lumina of the capillaries is regarded as evidence that the glands were actively releasing adrenal cortical hormone.

A marked storage of birefringent material, as well as a change in the appearance of the cells in the glomerular and reticular zones is described in castrated animals treated with 500 mg. of adrenotrophic hormone over a period of 20 days.

The thymus and sex-accessory glands of animals treated with adrenotrophic hormone showed responses which were proportional to the amount of adrenotrophe administered and, presumably, to the production of hormone by the stimulated cortices. The sex-accessories showed stimulation while the thymi were involuted.

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PLATE 1

EXPLANATION OF FIGURES

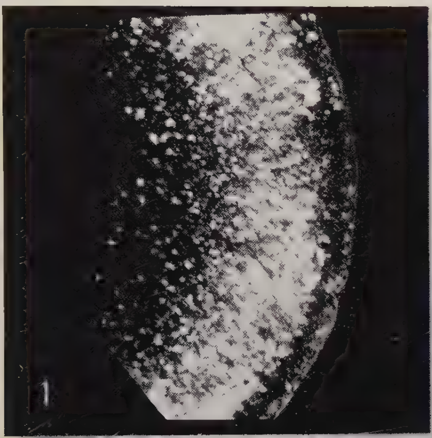
All photomicrographs at same magnification — approximately 45 diameters.

1 Section through adrenal cortex of a normal male. Reading from left to right can be seen the inner zone, the optically active zone, the optically inactive zone and the outer zone. Age at start, 28 days; weight at start, 70 gm.; age at sacrifice, 53 days; weight at sacrifice, 136 gm.; weight of adrenals, 36 mg.; weight of thymus, 256 mg.

2 Section through adrenal cortex of an untreated castrated female. Note relative loss of fine dust-like particles of birefringent material and the increase in the number of large birefringent globules. Age at castration, 28 days; weight at castration, 79 gm.; period of castration, 25 days; age at sacrifice, 53 days; weight at sacrifice, 138 gm.; weight of adrenals, 33 mg.; weight of thymus, 324 mg.

3 Section through adrenal cortex of a castrated male administered 400 mg. of adrenotropic hormone over a period of 20 days. The gross hypertrophy of the cortex, the loss of the fine dust-like particles of birefringent material and the absence of cortical zones is apparent. Age at castration, 28 days; weight at castration, 78 gm.; period of castration, 25 days; age at sacrifice, 53 days; weight at sacrifice, 130 gm.; weight of adrenals, 73 mg.; weight the thymus, 63 mg.

4 Section through adrenal cortex of a castrated male administered 500 mg. of adrenotrophic hormone over a period of 20 days. The gross hypertrophy of the cortex together with the marked storage of birefringent material is evident. Age at castration, 28 days; weight at castration, 66 gm.; period of castration, 25 days; age at sacrifice, 53 days; weight at sacrifice, 112 gm.; weight of adrenals, 71 mg.; weight of thymus, 46 mg.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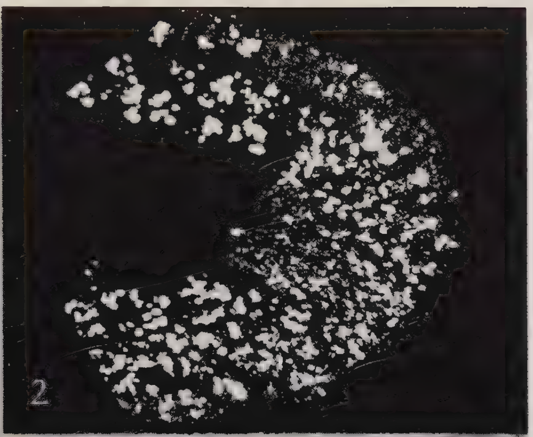
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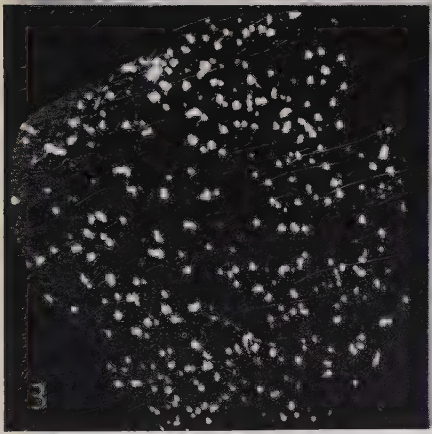
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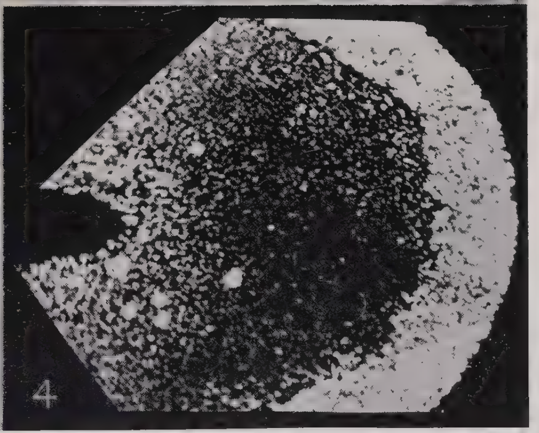
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OVULATION, MATURATION AND FERTILIZATION IN THE FOX

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FOUR PLATES (FIFTEEN FIGURES)

The reproduction of the dog early attracted attention because of the classical work of Bischoff (1845), but other Canidae have received little attention. In the course of a study of reproduction in the fox, observations have been made which are of special interest when compared with those of Bischoff and later workers on the dog. Emphasis has been placed in this communication upon polar body formation because several workers have reported that in the dog no polar bodies are given off until after ovulation, a situation that has been demonstrated for no other mammal. Rowlands and Parkes ('36) do not report on this phase of the reproductive processes of the fox because they lacked the facilities for securing the material. Taxonomically the fox is not a very distant relative of the dog, yet even a hasty examination reveals that reproduction in the fox differs in several respects from the dog.

MATERIALS AND METHODS

The animals used were silver foxes, Vulpes fulva, of the type raised commercially throughout the country, and were kept at the U. S. Fur Animal Experiment Station at Saratoga Springs, New York. Under ranch conditions the silver fox has a single heat period in the early spring, most of the animals mating in February. Each female is receptive for a period of 1 to 5 days, the usual length of receptivity being 3 days.

In the present experiments the males and females were kept in separate cages and a female was introduced into a male cage when vulvar examination indicated that she was approaching estrum. The animals were then kept under observation to record any mating or other behavior which might indicate the sexual condition of the female, and were separated after mating or after it became evident that the male would not attempt copulation. Females near estrum were tried daily with a number of males in order to lessen the possibility of missing the beginning of receptivity. This was thought necessary because of the monogamous tendency of the male fox. In this study receptivity was measured from the first copulation, and all other events in the cycle have been calculated using this as a reference point.

The tissues used in this study were obtained from thirty-three foxes by laparotomy or from animals killed by ether; all were fixed immediately in Bouin's fluid and slides were stained with Delafield's hematoxylin and eosin. Ova were not washed from the oviducts, but were sectioned while still in the tubes. Forty-four tubal ova were prepared in this way.

We wish to express our thanks to Charles F. Bassett, director, U. S. Fur Animal Experiment Station for his whole-hearted cooperation.

OVULATION

Two or 3 days before the first mating follicles destined to ovulate have already grown to a considerable size, about 4 mm., and contain a large antrum. The ovum and its surrounding cells are attached to the mural granulosa in conventional manner. The granulosa is thin, only a few cells deep, and many of the nuclei of the basal layer of cells are apical in position. Large follicles not destined to ovulate are easily distinguished at this time by the extreme thinness of the granulosa.

A noteworthy feature of the fox is the folding of the granulosa that occurs at this time and continues to be an important characteristic until the corpus luteum has formed. The granulosa appears to be thrown into small folds or ridges which in cross section give the appearance of tufts or villi, and which increase in size and complexity as the follicle matures and the corpus luteum forms. Many of the tufts include a large blood vessel. In fact, it is possible that at the time of estrum the unique enlargement of the blood vessels immediately underlying the basement membrane actually pushes the granulosa into folds which later grow into more complex tufts.

Folding of the granulosa of the dog has been mentioned by Bischoff (1845), Bouin and Ancel ('08), Van der Stricht ('08), and has been illustrated by Evans and Cole ('31) in a mature follicle, and by Arenas and Sammartino ('38) in an early corpus luteum of the dog. There is no folding in our fox material as great as that shown by Evans and Cole.

Some follicles rupture before or at the time of first acceptance, but this is not the rule. The unovulated follicle at this time may measure as large as 7 mm. in section; it contains a free floating ovum surrounded by an elongate spider web of attenuated cells, or an ovum completing its separation from the granulosa (figs. 1 and 4). The primary and secondary liquor folliculi are sharply distinct, but the separation between the two is not membranous. The folded nature of the granulosa is even more noticeable than in the proestrus follicle, and in many places small blood vessels have extended from the large blood vessels in the center of a fold into the spaces between the granulosa cells. It is apparent therefore that the granulosa is richly supplied with blood even before ovulation.

The tufts or folds into which the granulosa is thrown increase greatly in length and size at the time of first acceptance, sending out branches, especially in those follicles possessing the lace-like granulosa described below. These side branches consist usually of a core containing small blood vessels and delicate connective tissue, all surrounded by granulosa cells which may already have assumed luteal characteristics.

These folds of the granulosa play a leading part in the filling of the antrum by luteal cells. The folds grow rapidly toward the center of the follicle accompanied by blood vessels and luteinizing cells. The entire task of filling the antrum in some follicles is accomplished by the folds, whereas in other follicles there is considerable assistance from the granulosa and theca lying between the folds. The antrum does not fill completely until 2 or more days after ovulation, but there is considerable variation in this respect because there are two methods of corpus luteum formation, with combination or intermediate methods not uncommon.

One method is that just described in which, although the growth is rapid, the luteal cells have time to become roughly consolidated and organized. The second method is apparently achieved by the extremely rapid growth and branching of the folds of the granulosa. So rapid is this growth that the luteal cells appear to be unable to consolidate, which results in a very loose, diffuse corpus (figs. 5 and 6). Many of the cells rapidly become luteal in nature while others elongate or retain follicular characteristics only to luteinize later. This loose, open condition of the corpus is most noticeable about 1 day after ovulation, and it is soon obscured by the further growth and consolidation of the cells to form a compact corpus. This condition is found only in certain ovaries; the reason for the different rates of growth and luteinization in different animals is not known. Undoubtedly this open-meshed early corpus luteum is similar to the "lacework" luteal tissue found in occasional follicles of the bitch by Evans and Cole.

Ovulation in the fox is spontaneous. The material used in this study indicates that ovulation usually occurs late on the first day or early on the second day of acceptance. Only one of the six animals killed on the second day of heat had not yet ovulated, and every animal sacrificed later than the second day had ovulated. These results agree for the most part with those reported by the Russian worker Starkov (unpublished).

The journey of the ovum from follicle to oviduct is accomplished quite rapidly since no ova were found in the ovarian capsule despite the fact that a number of ovaries recently ovulated were sectioned while still enclosed in the capsule. The rapid movement of the ovum is a tribute to the efficiency of the fimbriated end of the oviduct which not only aids in this transport but also effectively aids in the occlusion of the slit which opens from the capsule into the abdominal cavity.

POLAR BODY FORMATION

A tightly fitting corona surrounds the ovum at the time of ovulation. The cells of the corona are neither as greatly attenuated nor the nuclei as conspicuously apical as in many other species (fig. 2). The ovum is elliptical in section, enclosed by a well-defined zona pellucida, and in the sections averages 104 × 76 micra. At one side of the ovum there is a large germinal vesicle which stands out conspicuously from the rest of the ovum because of the clear nature of its protoplasm and the distinct membrane that surrounds it. A few small dark spheres are always clustered at an indefinite point near the edge of the vesicle, and a few whisps of dark-staining fluid are usually present.

Ova found in the upper end of the oviduct have changed little in size or appearance from those fixed while floating free in the follicle (fig. 8). The corona, however, has contracted into a tight covering two or three cells thick. The germinal vesicle is still enclosed by a distinct membrane and polar body formation has not yet begun. Ova may descend more than one third the length of the oviduct in this condition.

The beginning of nuclear activity is characterized by the disappearance of the vesicular membrane and the appearance of numerous small chromatin masses within the vesicle. Ova in this condition were found in animals killed on the first and second day of mating.

The first maturation spindle soon forms (fig. 9), and is probably short-lived. Bishop ('42) has counted and illustrated sixteen tetrad chromosomes arranged on the metaphase plate of a first meiotic division, this number agreeing with the chromosome count which he made using testicular material. The first polar body is soon extruded and the remaining chromosomes, after condensing into a small sphere, separate again, becoming incorporated in the new second spindle. Both first and second spindles are perpendicular to the surface of the ovum at all times. Clear polar views of two second spindles have yielded chromosomal counts of 14 in both cases. This must be considered as the minimum number of dyads present, since one or two may have been obscured by overlying chromosomes.

Most of the ova recovered were in the stage possessing the first polar body and the metaphase of the second maturation division (fig. 10), and it is believed that the ova remain in this stage until fertilization. Frequently the first polar body divides before the second has been given off.

FERTILIZATION

The sperm penetrates the ovum when it has reached approximately the middle of the oviduct. Fertilized ova have been found which have not yet formed large male and female pronuclei in animals killed on the third and fourth day after the first mating, probably 2 and 3 days after ovulation. Shortly after penetration the male nuclear material is found as a small, compact sphere near one edge of the ovum. The fate of the tail of the sperm is not known since it has not been found either within the egg or among the corona cells. As soon as the second polar body is given off the maternal chromosomes condense into a small sphere of almost the same size and staining properties as the male nucleus, and it can only be distinguished by its position near the polar bodies.

One of the most interesting features of fertilization is that the sperm must penetrate through two or three layers of compact corona cells in order to reach the ovum, and that this corona may persist until after the pronuclei have formed (fig. 11). By the time the ovum has divided into the 4-cell stage, the corona has broken away and the zona pellucida has completely disappeared (fig. 12). A later stage sectioned in the uterus (fig. 13), although subjected to the same fixation and treatment, has a pronounced zona. This apparent discrepancy should be further investigated, but material fixed during the early divisions of the ovum has not been available.

DISCUSSION

Important differences between the reproductive mechanisms of the dog and the fox are: the shorter duration of receptivity in the fox, the occurrence of heat only once a year, and its restriction to a particular season. The dog probably differs from all other Canidae in the possession of two mating periods in a single year. The natural monogamous tendency of the wild male fox is being largely eliminated by careful selection of breeding stock and advanced breeding methods now being practiced on fur farms.

The mechanisms of ovulation, corpus luteum formation, and polar body formation are essentially the same in both animals. The unusual folding of the granulosa described for the dog is also found in the fox, although probably to a lesser extent. The time at which ovulation occurs, measured from the beginning of receptivity, is probably somewhat earlier than in the dog, but it is difficult to say definitely because there is little agreement as to the time of ovulation in the bitch (Whitney, '40).

The fox ovum is essentially the same size as that of the dog, although it tends to be slightly narrower.

The delay of polar body formation until after ovulation, heretofore demonstrated only in the dog, is also found in the fox. Both Bischoff and Van der Stricht ('23) have reported that the sperm may enter the dog ovum at any time after ovulation. On the contrary no sperm enter the fox ovum or even penetrate the corona until the second spindle stage has been reached.

SUMMARY

1. Large follicles of the fox are characterized by a folding of the granulosa even before ovulation.

2. These folds increase in size and complexity after ovulation and play an important part in the formation of the corpus luteum.

3. Ovulation occurs most frequently late on the first day or early on the second day of receptivity.

4. The first polar body is not given off until the ovum is well down the oviduct.

5. Fertilization occurs in the middle portion of the oviduct, probably 1 or more days after ovulation.

6. A tight covering of corona cells is retained around the ovum for a considerable time after fertilization.

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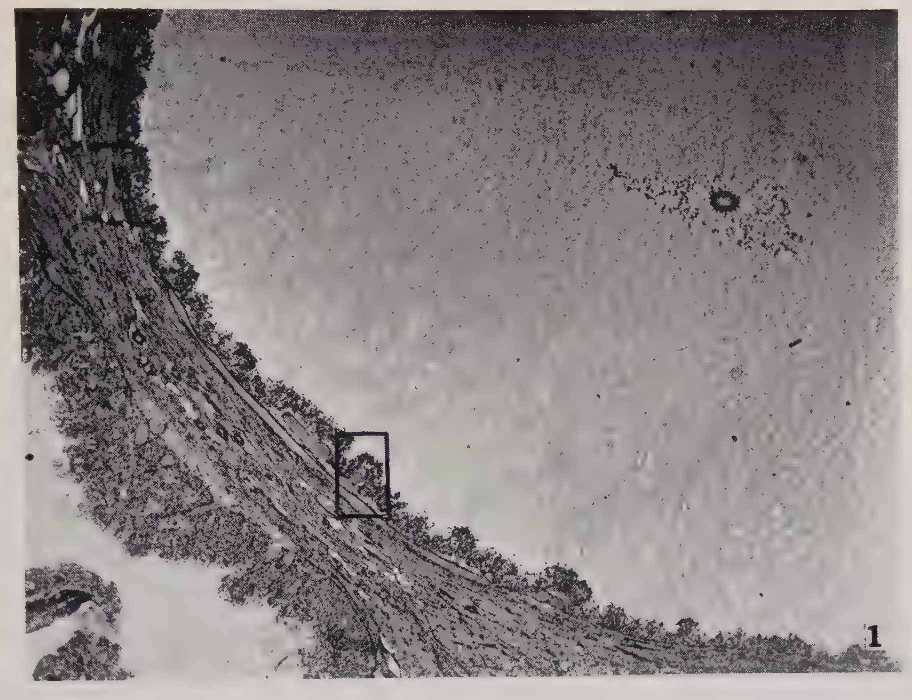
PLATE 1

EXPLANATION OF FIGURES

1 Follicle ready to ovulate. Ovum free from cumulus. Boundary between primary and secondary liquor folliculi clearly defined. The follicle in lower left hand corner ruptured while ovary was being removed. Second day of receptivity. Fox 62 B. × 12.

2 Detail of ovum shown in figure 1. × 170.

3 Detail of follicle wall marked by square in figure 1. Note folding of granulosa and numerous blood vessels. × 180.



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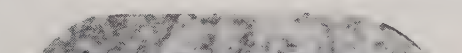

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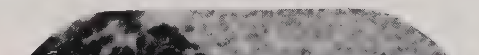

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PLATE 2

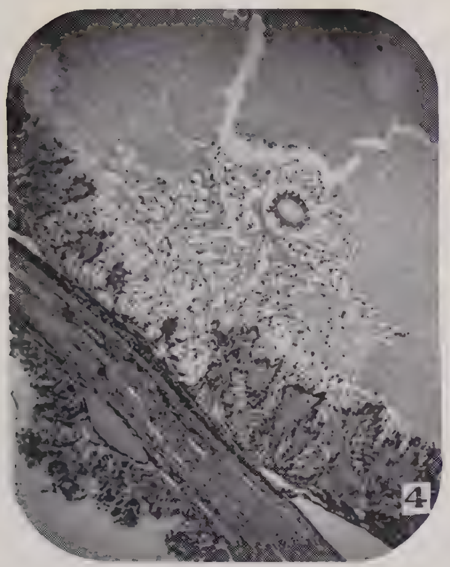
EXPLANATION OF FIGURES

4 Ovum separating from granulosa. The granulosa is affected locally down to the basement membrane. Note primary and secondary liquor folliculi and folding of granulosa. Second day of receptivity. Fox 62 B. × 38.

5 Early corpus luteum of diffuse type. Third day of receptivity. Fox 80 B. × 15.

6 Detail of corpus luteum shown in figure 5. Compare loose tissue arrangement with figure 7. × 175.

7 A compact corpus luteum. Figures 6 and 7 represent extremes of the two types of luteal structure. Third day of receptivity. Fox 779 F. × 175.



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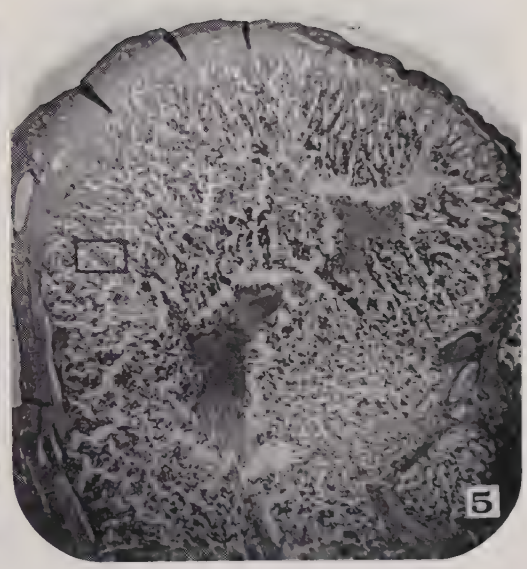

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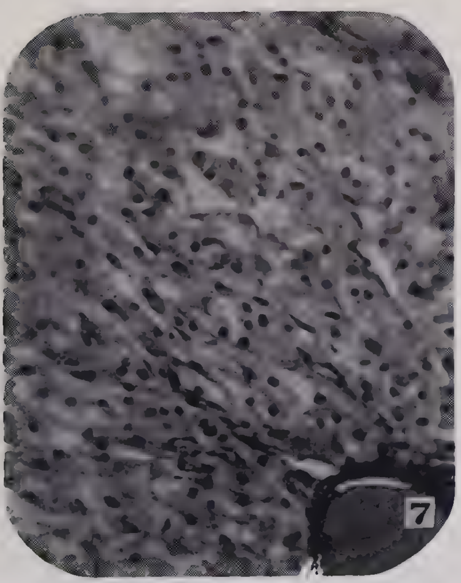

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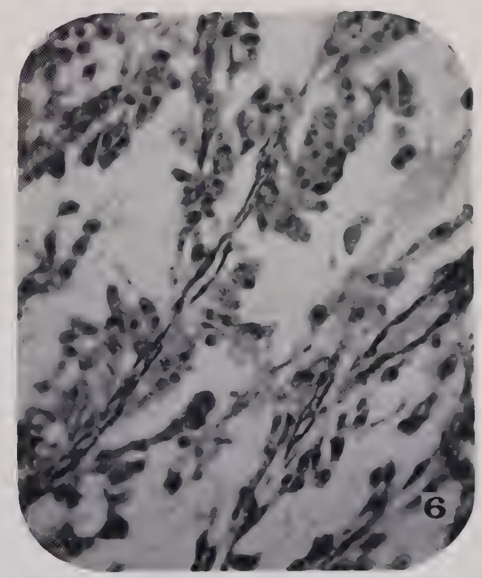

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PLATE 3

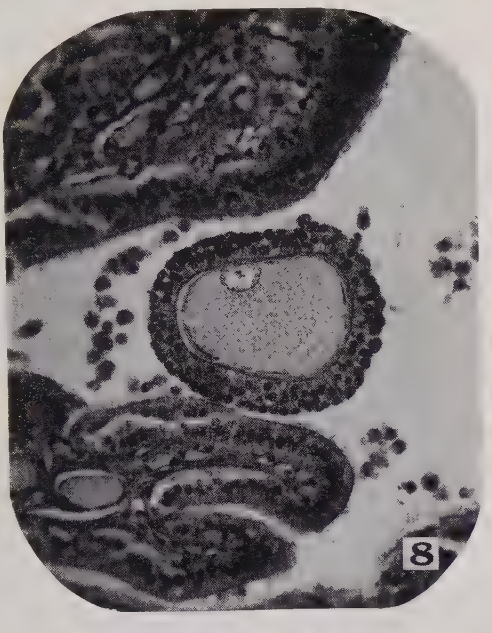
EXPLANATION OF FIGURES

8 Ovum that has descended approximately one third the length of the oviduct. Note intact germinal vesicle and absence of polar body. Second day of receptivity. Fox 741 F. × 200.

9 Metaphase of first maturation spindle. Third day of receptivity. Fox 80 B. × 1000.

10 Second maturation spindle. Second or third day of receptivity. Fox 50 C. × 910.

11 Fertilized ovum showing one of two pronuclei. The corona radiata is persistent. Note sperm head touching zona at 9 o'clock. Fourth day of receptivity. Fox 170 B. × 340.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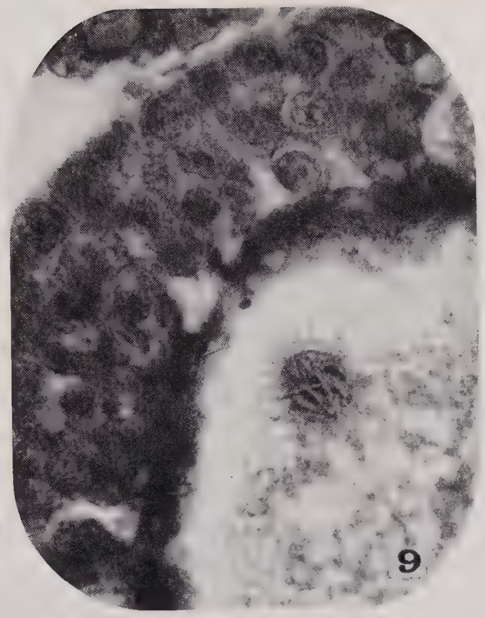
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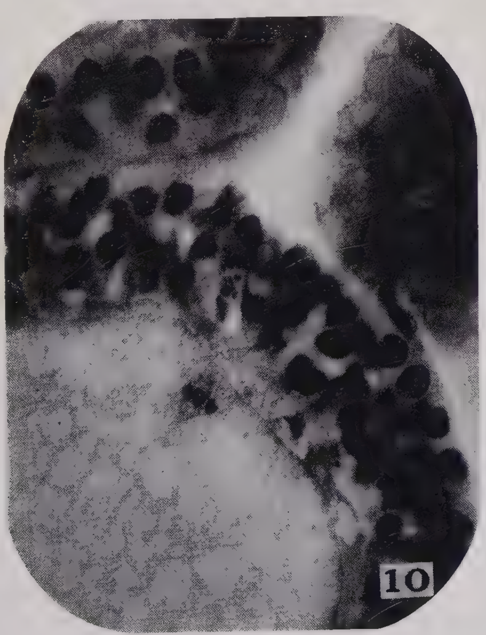
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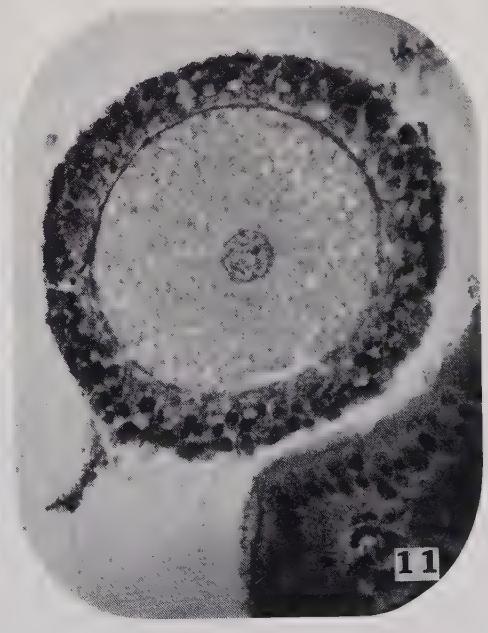
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PLATE 4

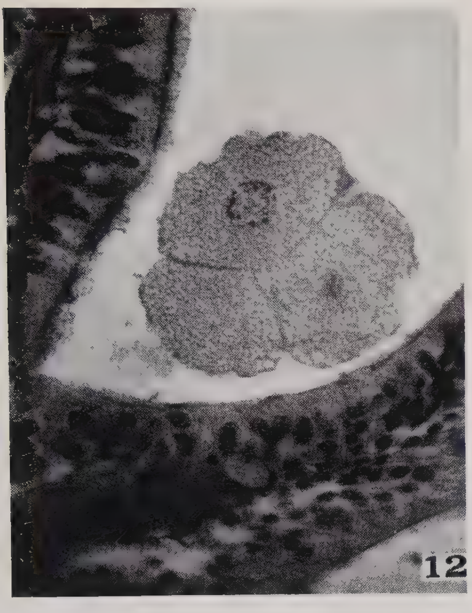
EXPLANATION OF FIGURES

12 Four-celled ovum in lower third of oviduct. Note absence of zona pellucida. A large polar body lies just below the plane of focus at lower left. Five days after first mating. Fox 202 C. × 410.

13 Free blastocyst in uterus. A distinct zona pellucida is present. Nine days after first mating. Fox 779 F. × 410.

14 Diagram showing the progress of polar body formation in the fox and the approximate region of the oviduct at which the various events occur. Numbers refer to the photographs in plates 3 and 4 which illustrate the same stage of development.

15 Section through ovary showing unusual everted type corpus luteum. Only one such corpus was observed. Ovum visible in oviduct. Third day of receptivity. Fox 1021 F.



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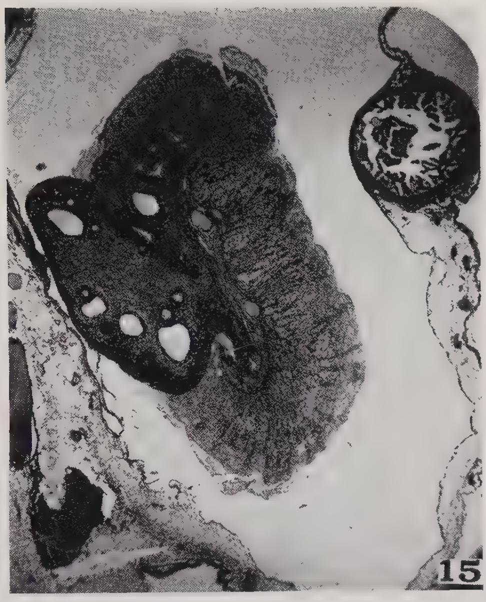

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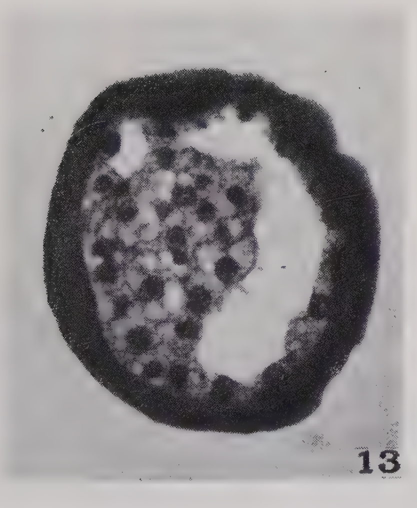

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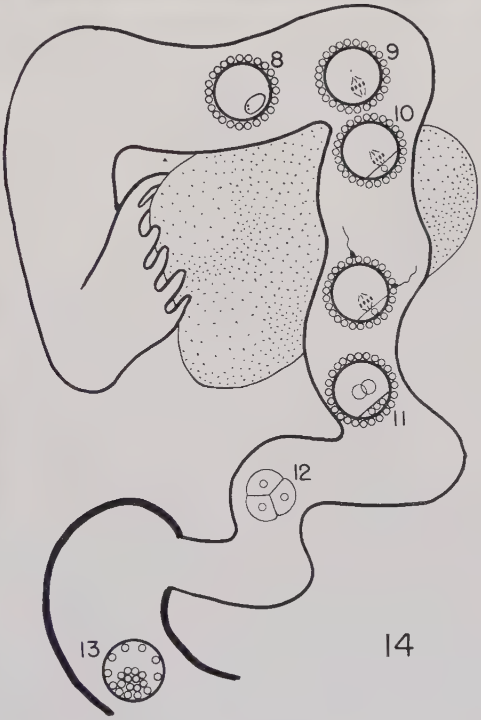
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ON THE PRESENCE OR ABSENCE OF NERVES IN THE UMBILICAL BLOOD VESSELS OF MAN AND GUINEA PIG

MARY SPIVACK
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SIX FIGURES

The presence or absence of nerves in the umbilical cord is an old and still mooted question. It may appear strange at first thought that the presence of innervation in such a vital structure as the umbilical cord should even be questioned. However, closer acquaintance with the finer anatomy and histology of tissues reveals that there are other important structures in the human organism in which nerves have not been demonstrated with certainty up to the present time. The innervation of the cerebral blood vessels might be mentioned as a classic example of such a disagreement among the authorities in the field of neuro-histology. The discussion pertaining to the innervation of the umbilical cord began in the time of Galen; it has occupied the minds of scientists during the past 300 years. The dispute was lively at one time, lagged or became quiescent at other times, only to be revived in the present century. That the problem is a difficult one is attested by the numerous controversial contributions. It is a known fact that the demonstration of nerves in the blood vessels is not an easy technical procedure; they are especially difficult to detect in small capillaries. The considerable experience required in diagnosing the finer branches of the peripheral nervous system also adds to the task. Not only is it easy enough to miss the presence of fine nerve fibers, but it is easier yet to diagnose one when it is not present. In a number of instances artefacts and connective tissue fibers have been described by authors as nerves. The present author tried assiduously to avoid the pitfalls of some previous investigators, and hopes that she has succeeded in part at least.

I wish to acknowledge gratefully my indebtedness to Dr. O. F. Kampmeier, Head of the Department of Anatomy, for the invaluable help received throughout this study.[1]

HISTORICAL DATA

Even as early as the second century Galen occupied himself with the problem of innervation of the umbilical blood vessels; his findings were of negative nature. During the past two centuries numerous papers, some pro, some con, were written concerning this question, but generally very little information about the subject can be gathered from obstetric textbooks, especially those printed in the English language. A complete review of the literature was given in 1836 by Schott in his detailed monograph. The literature prior to Schott's publication is omitted here because the work of the earlier investigators lacked the scientific approach and technique. Some of them based their opinions upon general physiological considerations and upon the authority of other authors, and a few even upon mere superstition. For example, it was believed by some that nerves are not necessary for the life of the fetus, that they have no purpose for the baby in utero, therefore they must be lacking. The insensibility of the umbilical cord to pain seemed to some writers proof of the absence of nerves, while the unconfirmed observation of the contraction of the abdominal muscles in the newborn upon cutting the cord led others to accept the presence of nerves in this structure. One writer queried how would maternal impressions be transmitted to the fetus if not by a nervous path? All of this is quoted from Schott.

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[1] The human material was obtained from the Obstetrical Department of the Women and Children's Hospital of Chicago. I take pleasure in thanking the members of the medical staff for their cooperation in securing the specimens.

Home (1825), according to Bucura ('07), was the only writer of the early period to utilize the microscope (actually he presented drawings of unstained preparations magnified four to ten times) in an endeavor to prove his contention of the presence of nerves in the placental vessels and umbilical cord of man. Schott's own contribution consisted in demonstrating nerves by means of anatomic dissection in the abdominal portion of the umbilical vessels. The nerves of the vein, according to Schott, originate from the left hepatic plexus and can be traced to the umbilicus. The umbilical arteries also possess nerves which take their origin from the plexus uterinus lateralis in the female, and in the male from the rectal plexus. These nerves pass the umbilical ring and can be traced along the cord for a distance of somewhat more than 1 inch beyond the navel (man). Schott did not give microscopic evidence for his contentions.

Soon afterwards (1837), Valentin corroborated and even extended the findings of Schott. He could trace the nerves for about 3 to 4 inches (approximately 8 to 11 cm.) outside the navel. That he made his observations with the microscope on unstained material may be gathered indirectly from his statements.

From then until Fossati published his work in 1905, several articles either entirely negative, or only corroborating the findings of Schott and Valentin, were added to the literature. Thus, Virchow (1851), Gimbert (1865), and Tait (1876) could find no nerves in the umbilical cord, although Gimbert qualified his conclusions by stating that the intra-abdominal portion of the umbilical blood vessels possesses vasomotor nerves. Kölliker (1879) found nerves in the proximal part of the umbilical cord of man and animals, but did not find them in the distal portions of the cord and in the placental vessels, thus confirming the findings of Valentin. In view of the well-known great contractility of the umbilical blood vessels, the absence of nerves in their major portion was of considerable physiological interest to Kölliker. Minot (1892) accepted the view that there are no nerves in the distal portion of the umbilical cord. Lochman ('00), on the other hand, failed to find nerves in any stretch of the umbilical and placental blood vessels, and Webster in his obstetric textbook ('03) adopted the opinion that a nerve supply in the vessels of the cord did not exist.

Fossati ('05, '06 and '07), already mentioned, described a peculiar network in the chorionic villi of the placenta which he considered to be nervous, or at least to resemble nerve tissue by its staining reactions. His findings were strongly disputed by Bucura ('07 and '08), who in his many years of study of the human umbilical cord and placenta could not detect nerves in either of these structures.

Goenner ('06) was able to corroborate macroscopically Schott's findings. He made an attempt to study the umbilical cord microscopically, but arrived at no definite results due to lack of specific stains.

There was some lull in the writings on this subject until Schmitt published his work in 1922. Although he used such a reliable method as Stöhr's modification of Schultze's silver technique, he could reveal no nerves in the umbilical or the placental blood vessels.

Argaud ('22) is one of the few authors of the present century whose labors were rewarded by what he believed to be positive microscopic findings. By using the gold chloride method of staining he found in the wall of the umbilical blood vessels at all levels of the cord a rich network which he interpreted as nervous. He stated that he had even been able to demonstrate nerve-endings.

Mabuchi ('24) claimed that he could demonstrate non-myelinated nerves in the umbilical and placental blood vessels and in Wharton's jelly of man by modifying Cajal's method. Nerve endings, seen in the media, were exclusively of the free type. Yet Stöhr, Jr. ('28) is certain that Mabuchi's findings are artefacts, and he says that he himself could detect nerves neither in the umbilical nor in the placental blood vessels. Runge, Baur and Hartmann ('28) also did not succeed in showing nerves in the umbilical cord, although they employed modern methods of staining.

Schröder ('30), summarizing briefly in v. Möllendorff's "Handbuch der mikroskopischen Anatomie des Menschen" the status of our knowledge concerning the innervation of the umbilical cord, makes the statement that thus far nerves have not been demonstrated definitely in this structure. A year later Martha Dancz ('31) asserts that the umbilical blood vessels of man are richly supplied with non-medullated nerves which are of sympathetic origin. She also claims the presence of nerve endings in all the three layers which she recognizes in the umbilical blood vessels. She ascribes her good luck to longitudinal sections and to the silver pyridine stain of Cajal for frozen sections. Her work caused a lively polemic between her and Stöhr, Jr., who in 1932 emphatically and caustically denied the scientific value of Dancz's findings, considering them as artefacts. Stöhr steadfastly maintained that in his hands the umbilical blood vessels of man have not revealed an unquestionable nerve supply, although he made a consistent effort to find it. (In 1932 he made such a statement regarding placental vessels and again in 1938 regarding the umbilical ones.)

Ooi ('34) is emphatic about the existence of non-medullated nerves in all umbilical blood vessels of man, rabbit and guinea pig. He found nerve bundles and their branches "perivascularly," and in the "adventitia" and media of those vessels. He even saw a variety of nerve-endings and microganglion cells, the latter in the "adventitia."

In the same year ('34) Fritz, in a study of ninety-five human umbilical cords, came across the presence of small ganglion cells in one specimen, in which they occurred in the umbilical vein and in Wharton's jelly 4 mm. outside the navel.

As late as 1940 Scalzo, in a continuation of work previously published ('39), described in the human cord an abundant nerve-supply which consisted of non-myelinated nerve trunks and numerous anastomosing branches. Upon using the Gross-Bielschowsky silver stain, he observed them as a fine network in the muscular tunic, especially in the proximal end of the cord. No nerve endings were detected.

After surveying the literature, it can be said that the majority of modern authors gave no consideration to the presence and relations of the nerves in the abdominal portions of the umbilical vessels. Those few of the older authors who did consider them made their assertions regarding the presence of nerves exclusively upon macroscopic dissection, a method which is almost worthless in the diagnosis of fine nervous structures. In the published papers the present writer has not seen a single microscopic illustration of nerves in the abdominal umbilical vessels.

MATERIAL AND TECHNIQUE

The author's material consisted of umbilical cords of man and guinea pigs at or close to term. The main volume of work was done with the methylene blue dye upon the guinea pig. Of all variations of the methylene blue methods, the immersion technique proved to be the most satisfactory. The guinea pig material could occasionally be stained with fair results by perfusion also, while the human material was well adapted to the immersion method only. A $\frac{1}{100}$% solution of Grübler's methylene blue, dissolved in normal saline prepared with double distilled water, was used throughout this work. Methylene blue was dissolved by placing the dye in the normal saline solution in a hot water bath for about 5 to 6 minutes. When the solution of methylene blue was cooled to 37°C., the tissues to be stained were placed in it and kept in the incubator at the above-mentioned temperature for varying intervals of time.

The human umbilical cords prior to staining were cut at various levels: near the navel, at the middle, and near the placenta. The time of staining varied from 15 minutes to 1 hour. The progress of staining was controlled under the microscope after squeezing the relatively thick tissues between two microscopic slides. After the completion of the staining the tissues were fixed in an 8% solution of ammonium molybdate in which they remained overnight at room temperature. Next day the specimens were washed in three changes of distilled water for about 1 hour and dehydrated in three changes of absolute alcohol for another hour, cleared in oil of wintergreen for about 5 minutes, and mounted upon the slide with Canada balsam. For the first 3 to 5 minutes of dehydration the tissues were compressed between two slides in order to obtain and retain their thinning.

When animals were used the whole umbilical cord together with the continuation of its blood vessels within the abdominal cavity and the fetal urinary bladder were dissected out from the adjacent tissues, and stained immediately with the freshly prepared solution of methylene blue, as above described. A piece of navel skin was usually retained in the specimen as a landmark, delineating the cord proper from the abdominal umbilical blood vessels. Other methods, such as the silver pyridine method of Cajal for frozen and paraffin block sections, Ranson's modification of the silver pyridine stain, and Davenport's silver paraffin method for mounted sections were employed as controls only.

As was mentioned before, the pregnant guinea pig proved to be a most satisfactory animal for this study. Eleven litters were employed in the experiments; all of the litters but two were subjected to the supravital methylene blue dye, and three fetuses to the silver method of Schultze-Stöhr.

Four human cords, cut at different levels — navel, middle and placental — were stained supravitally with methylene blue, either in toto or after dissection of the individual blood vessels, as described above. One human specimen, obtained from a full-term still-born fetus, and consisting of the umbilical cord, urinary bladder, and the abdominal portions of the umbilical arteries and vein, was stained according to the Schultze-Stöhr silver technique.

The author spent a great amount of effort in perfecting herself in the technique of methylene blue staining, during which other vessels besides those of the umbilical cord were so treated in order to become critical of its comparative behavior. Thus it was possible to demonstrate nerves with the methylene blue dye in the blood vessels of various organs and tissues with great frequency if not regularity. By demonstrating nerves in the blood vessels of various organs the author endeavored to obtain proof of sufficient technical preparedness to meet the requirements of the particular problem on the innervation of the umbilical blood vessels.

OBSERVATIONS

In none of the umbilical cords proper, no matter whether of man or of animal, and irrespective of the type of stains employed, was the author able to detect nerves or structures resembling them. But when the study was extended to the abdominal portions of the umbilical blood vessels, unmistakable nerves were found in them. The methylene blue dye was the first to reveal this fact; corroborating evidence was furnished by the Schultze-Stöhr sodium hydroxide silver stain. Thus in all eleven litters of guinea pigs nerves were found in one or the other or both internal umbilical arteries and in the inner portions of the umbilical vein. The picture was at times very striking. That part of the abdominal umbilical blood vessels which showed the stained nerves stood in sharp contrast to the pale, faded appearance of the nerveless cord itself. This comparison of both external and internal stretches of the umbilical blood vessels on the same slide was made possible by the supravital methylene blue method upon "squeezed" preparations, which formed the technical basis of this work. Unfortunately, the human internal umbilical vessels could not be studied by the supravital method, since only experimentation permits such a procedure. But the only specimen obtained from a still-born full-term baby revealed strikingly the presence of a rich supply of nerves in the abdominal portions of each of the umbilical blood vessels, when the Schultze-Stöhr silver stain was applied. The advantages of the "squeezed" preparations are more evident when the methylene blue dye is employed. This stain, unlike the silver, does not thicken the specimen appreciably. Hence, large stretches of tissues can be traced under the microscope and studied; it permits also an easy orientation in anatomic relationship. In the "squeezed" preparation the transition between the blood vessels of the cord and the abdominal umbilical vessels is readily seen with the naked eye (fig. 4). The low power of the microscope brings out sharply the tinctorial differences of the structures.

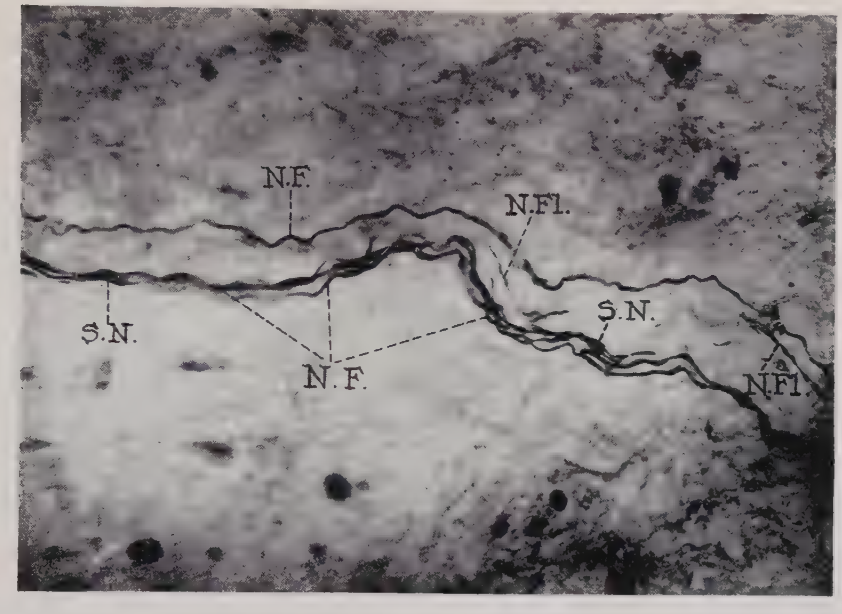

Fig. 1 Microphotograph of an abdominal umbilical artery of a guinea pig fetus, Schultze-Stöhr silver stain, × 500. N. F., nerve fiber; N. Fl., nerve filaments; S. N., nucleus of Schwann's cells. The varicosities of the nerve fibers are distinctly seen. Schwann's nuclei are scarce but can be detected plainly.

Microscopic description. The abdominal umbilical arteries of the guinea pig are unevenly supplied with nerve trunks and nerve fibers, which in some areas, especially in the adventitia are so abundant as to form coarse plexuses, while in many other areas the most careful search did not reveal them. The farther away from the navel (that is, the nearer the hypogastric arteries) the richer was the nerve supply. The larger nerve trunks ran parallel to the long axis of the vessel, giving off branches in acute angles, which crossed each other at some points to form wide meshes. The nerves were of the non-medullated variety. They had a wavy, spiral course, and their axons, when stained supravitally, could often, but not always, be traced as long threads for a distance of several microscopic fields. When the Schultze-Stöhr silver stain was applied the axons were deeply impregnated and appeared as distinct uninterrupted threads, running parallel to one another (fig. 1). In the abdominal umbilical arteries the nuclei of Schwann's cells were hard to detect when stained with the methylene blue, but they stained readily and sharply

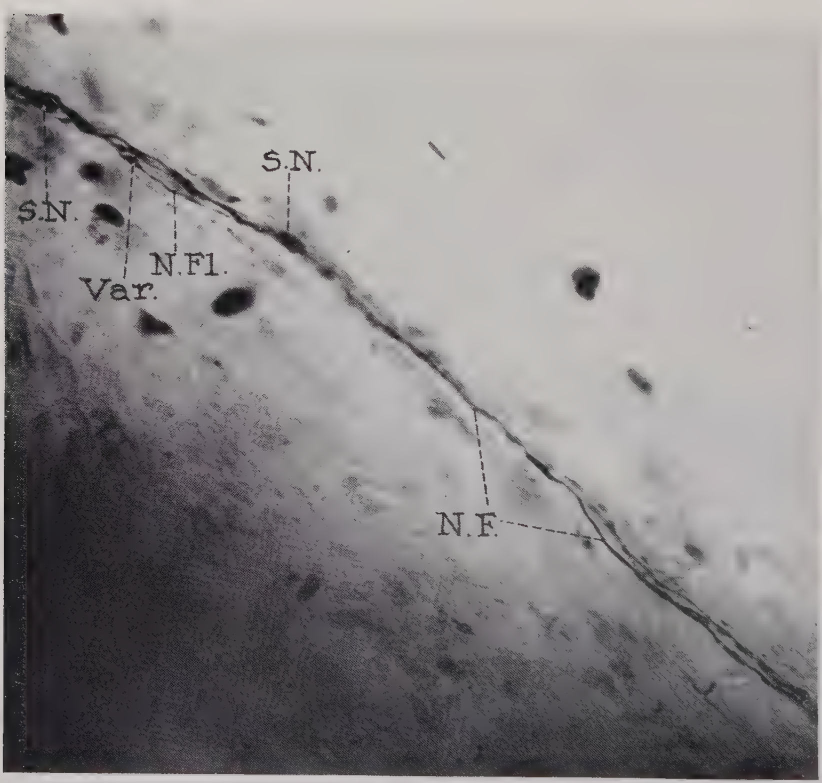

Fig. 2 Microphotograph of an abdominal umbilical artery of a guinea pig fetus, Schultze-Stöhr silver stain, × 400. N. F., nerve fiber; N. Fl., nerve filament; S. N., nuclei of Schwann's cells; Var., varicosity. The beading is seen at the right corner of the section. The Schwann's nuclei are marked.

with the silver method of Schultze-Stöhr. When detectable, they appeared as oval structures, situated alongside the axon, at uneven intervals and several times thicker than the axon itself (fig. 2). The diagnosis of a nerve fiber was easier with the Schultze-Stöhr stain than with the vital method, but its tracing for long distances was impossible.

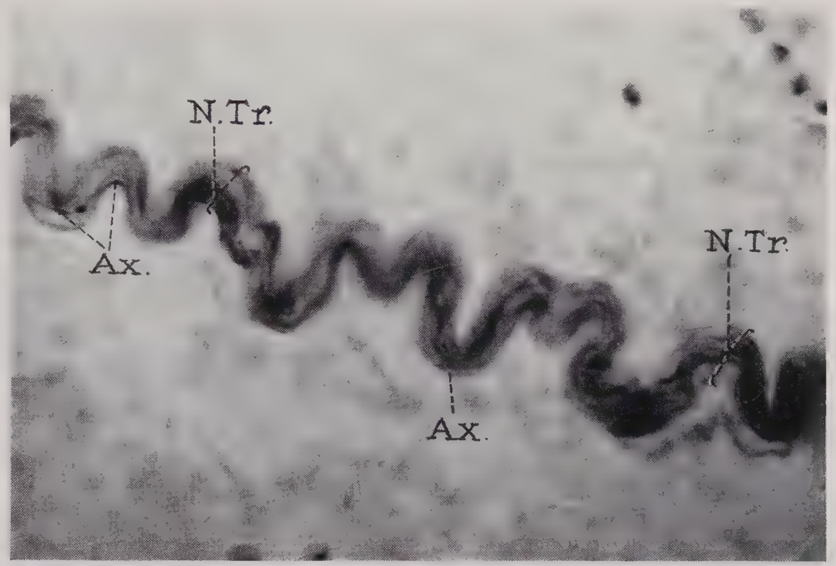

Fig. 3 Microphotograph of a piece of adventitia from the abdominal portion of the umbilical vein of a guinea pig fetus, methylene blue stain, × 500. N. Tr., nerve trunk; Ax., axon. The axons stained unevenly and photographed with difficulty, because of the thickness of the section. However, they can be discerned in some areas due to fibrillations of the trunk and the presence of characteristic varicosities.

The abdominal portion of the umbilical vein revealed a similar abundant supply of nerves, the main trunks of which were thick and wavy. Although the axons often stained unevenly and imperfectly with the vital stain, their recognition yet was possible due to their distinct fibrillation and the presence of varicosities (fig. 3). Checking with the Schultze stain whenever feasible clinched the diagnosis in doubtful cases. In general it can be said that the distribution of the nerve supply in the abdominal umbilical vein in the guinea pig does not differ in any way from that of the abdominal umbilical arteries.

On the other hand, the blood vessels of the umbilical cord proper, which in the guinea pig, unlike the human, are five in number (fig. 4), and two of which separate from the main trunk in the upper third of the cord to distribute themselves upon the fetal membranes, appeared to be completely nerveless when viewed under the microscope. The methylene blue stained the umbilical vessels homogeneously exhibiting no special affinity for any of its components, and giving a hue which is duller and paler than in the neighboring abdominal vessels.

As already stated the present observer did not see nerves in the human cord. The negative results were obtained by supravital staining with methylene blue, by Schultze-Stöhr's method, and by the above-mentioned variants of the silver technique. But again quite different results were achieved when the study was extended to the abdominal segments of the vessels, with the use of the silver method of Schultze-Stöhr.

Several small pieces of abdominal umbilical arteries and vein, picked at random from various levels of the human stillborn, revealed an abundant supply of non-myelinated nerves. Fibers, consisting of several typical axons, with their classic varicosities and distinct Schwann's nuclei were readily detected. The nerves in man, as in the guinea pig, ran a course parallel to the long axis of the artery or vein; frequently they divided dichotomously at one point, their branches doing the same thing at another until they thinned out into very fine nerve-filaments consisting of a naked axon only (fig. 5). From the study of this case alone it can not be said definitely at which

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Fig. 4 Photograph of the umbilical cord with all its abdominal relations and the urinary bladder from a guinea pig fetus, methylene blue, stained as a whole, × 2. U. V., umbilical vein (abdominal portion); U. V.$_{1}$, umbilical vein (after passing the umbilical ring); U. A., U. A., abdominal umbilical arteries; U. A.$_{1}$, U. A.$_{1}$, umbilical arteries (after passing the umbilical ring); C. D., blood vessels that there were traced from the navel to the mesentery of the small intestines; C_1 D_1, the same vessels outside of the navel; they separate from the main trunk of the umbilical cord at its upper third and distribute themselves upon the fetal membranes; E., urinary bladder; Sk., navel skin.

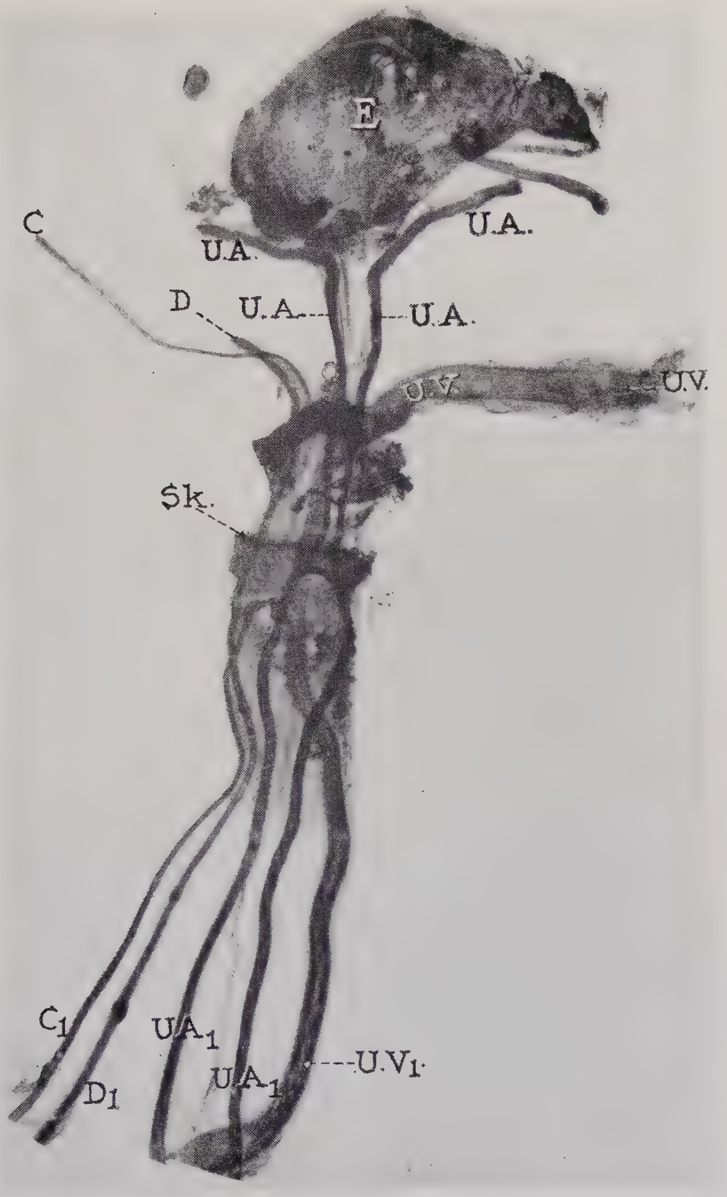
E
C
U.A.
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Figure 4

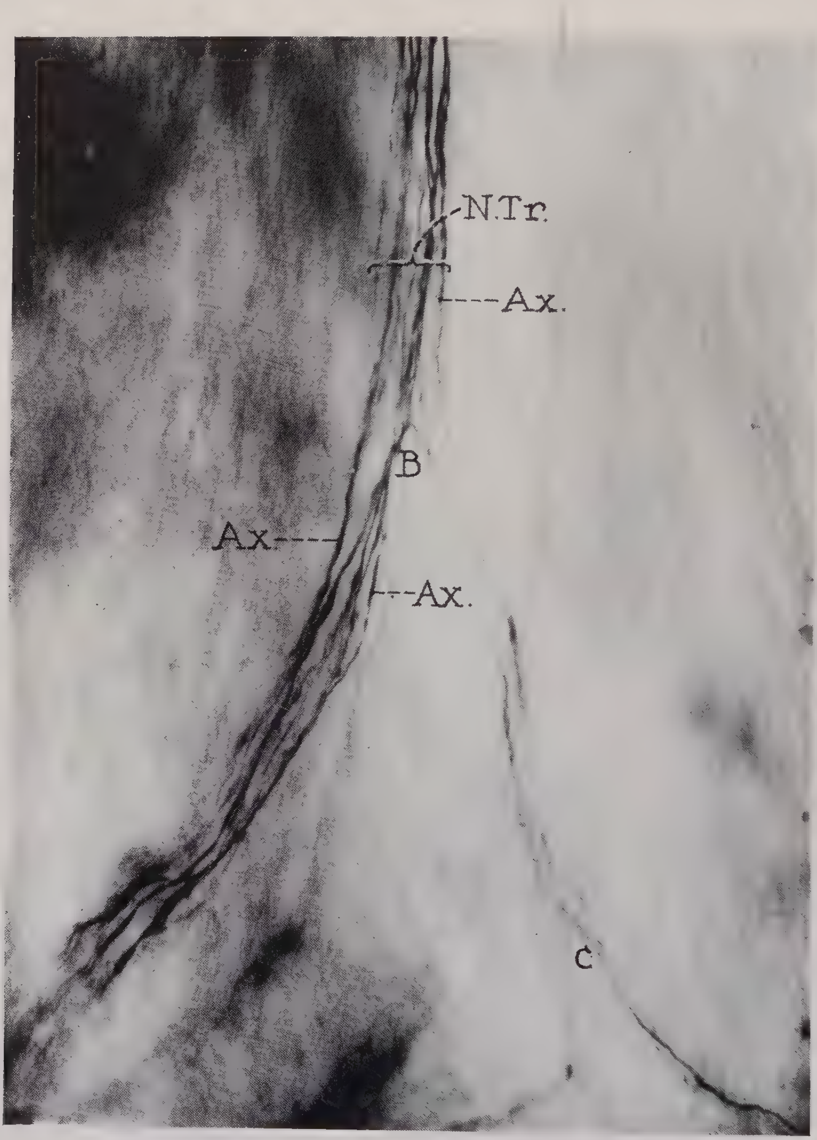

Fig. 5 Microphotograph of a piece of adventitia from an abdominal umbilical artery of a human fetus at term, Schultze-Stöhr silver stain, × 400. N. Tr., nerve trunks; Ax., axon. The main nerve divides dichotomously at B, and one of its branches divides at C. The varicosities are well exhibited. The paler appearing axons are out of focus in the thick section.

stretch along the abdominal umbilical vessels the nerves first appeared, because no effort was made to establish the relationship of the pieces of tissue toward the navel.

One other interesting observation should be recorded. In the small blood vessels of the human fetal membranes stained with methylene blue, there existed a network, the origin of which is hard to establish (fig. 6). The individual meshes of this network ran a longitudinal course and were grossly parallel to the long axis of the vessel. The network consisted of fine, wavy, beaded or varicosed threads. At some places the transverse junctions forming the meshes disappeared, leaving only the fine fibers that take the longitudinal course. The picture of such an arrangement resembles somewhat that described in the guinea pig by Busch ('29) and interpreted by him as of nervous origin.

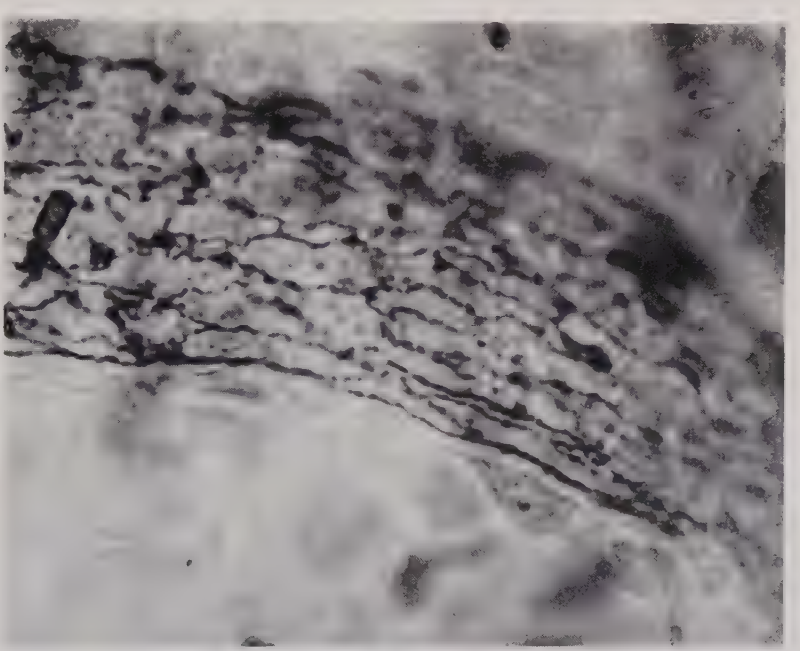

Fig. 6 Microphotograph of the surface view of a haemal capillary in the human fetal membranes, methylene blue, × 1000. Note the varicosities and fine waviness of the fibrils which form the perivascular network.

Ordinarily the guinea pig cord, like the human, is devoid of nutrient vessels. But exceptions are seen in both species. In the guinea pig, in one case only, the author saw fine, beaded, wavy lines forming a meshwork in some of the nutrient vessels. Nuclei of Schwann's cells were not seen, and no connection with a definite nerve fiber or nerve trunk could be established in these networks on capillaries in the fetal membranes of man, or in the nutrient vessels of the umbilical cord of the guinea pig.

DISCUSSION

After a careful study of the literature on the innervation of the umbilical cord, the impression remains that no undisputable proof of the presence of nerves has been furnished by those who claimed positive findings. The main weakness of most of the papers consists in the lack of adequate illustrations, only a few of the writers presenting microphotographs, while the majority are content with drawings. And while in some of the drawings the disputable structures more or less resemble nerve-elements, they seldom do so in the microphotographs. So unsatisfactory are the majority of the illustrations that one of the authors (Ooi), who arrived at conclusions similar to those of another (Mabuchi), has not been fully satisfied with the illustrations of his colleague. Stöhr went further, for he did not recognize the nervous nature of Mabuchi's findings at all. Ooi himself did not furnish irreproachable illustrations. In no instance did be demonstrate nuclei of Schwann's cells in questionable fibers. There is some resemblance to a nerve trunk in his microphotograph 2, in which he designates a nerve bundle in the "perivascular" tissue in the guinea pig. He gives no indication whether he speaks of an artery or of a vein, and gives no magnification. What he figures as a "microganglion" cell can hardly be recognized as such. The illustrations of the human material are the least convincing. Neither has M. Dancz, who received her full share of criticism from Stöhr, convinced the present author that her only microphotograph presents a nerve bundle.

However, a resemblance to nerve fibers was found in some of her drawings.

Fritz's findings of small ganglion cells, if they be accepted as such, in the umbilical vein and in Wharton's jelly in one case only in the material of ninety-five human cords may represent an anomaly. Argaud, Mabuchi, Fossati and Scalzo did not avail themselves of microphotographs at all. The present writer is fully aware of the difficulties of photographing nerves in thick sections, but she also is cognizant of the limitations of drawings, which needless to say do not reproduce a picture as faithfully as microphotographs do, because of the intruding personal element in interpretation.

Several of the authors have not demonstrated an intimate knowledge of the structure of the umbilical blood vessels and their peculiarities. Thus they (for example, Argaud, Ooi, Dancz) speak of the distribution of nerves in the "adventitia," as if this layer really existed in the external umbilical vessels of man. In man and guinea pig the umbilical vessels of the cord itself do not possess an adventitia, a fact known to some students of the cord. Unlike the external portion, the abdominal stretches of the umbilical vessels do have an adventitial layer like any other blood vessel of the same caliber. Lest it escaped the reader, the present author calls attention to the fact that her description of nerves in the adventitia pertains only to the intra-abdominal portions of the umbilical vessels.

It is regrettable that no truly specific nerve stains are in existence at the present time. It is true that both cardinal nerve stains (silver and methylene blue) demonstrate excellently nerve structures, but they stain to some degree other tissues also, and considerable skill is required to differentiate one from another. Needless to say many silver methods stain reticular tissues alongside with nerve elements, and the existence of the so-called argyrophil fibers is not a matter of dispute. The vital methylene blue dye, although having a pronounced affinity for nerve tissue, stains also muscle, fat and connective tissue. That reticular tissue can be stained by this dye is stated by Weddell and co-workers ('40). Such accusations that a stain is ''capricious'' or ''unreliable'' are only admissions of the fact that the mechanism of staining is not understood in its finer details. The Russians, who have contributed considerably to the perfection of the methylene blue staining method, made an effort to throw further light upon the mechanism of staining with this dye. Schabadash ('35) casts some doubt upon the principles laid down by Ehrlich himself, the discoverer of vital staining with methylene blue (1885). He disputes that oxygen saturation of cells and tissues occurs in the process. He maintains that mobilization of hydrogen atoms, for which methylene blue has a great affinity, is a very essential part of the procedure.

In the present material, it was frequently seen that nerve fibers may have stained very sharply in one field, only to appear pale, almost colorless, in an adjacent one, as if some unknown factor interfered with the staining. Even in the silver impregnation methods the affinity of the stain toward identical anatomical elements varied considerably in the same preparation. Occasionally a poorly stained or even a non-stained nerve bundle could be recognized by its morphological characteristics and by its direct continuity with a sharply stained one. When the umbilical cord is subjected to various methods of nerve staining, the same difficulties are encountered and, as some authors, such as Ooi, believe, those difficulties might even be augmented by the presence of Wharton's jelly. Ooi was inclined to consider that substance as a hindrance, at least, to silver stains. It can be said, however, that the absence or reduction of the jelly around the placental vessels do not make the demonstration of nerves in the placenta any more certain than in the cord itself.

When studying the innervation of the blood vessels, the observer must rely largely upon the morphological features of the questionable structures, and consider the tinctorial characteristics only as an adjuvant feature of diagnosis. Although the classic characteristics of a nerve fiber are well known, the author has not found that uniform criteria were applied in the diagnosis of questionable structures. In the work presented here a fiber was considered to be nervous if it showed the characteristic thickenings or varicosities in its course, and if it possessed the nuclei of Schwann's cells. Since the latter are widely and irregularly spaced, the nervous nature of a questionable structure was established only if it could be traced into continuity with a nerve trunk or another definite nerve fiber. The presence of Schwann's nuclei is a positive sign of a nerve fiber. The varicosities and beading, although suggesting an axon, do not necessarily prove the nervous nature of a structure, since fine connective tissue fibers may under certain conditions possess these features (cf. Maximov, in v. Möllendorff's ''Handbuch der mikroskopischen Anatomie des Menschen,'' II/I, S. 248, '28). Because reticular tissue is argyrophil, the difficulties encountered in the interpretation of silver stained preparations can be readily understood. Beech and Davenport ('33) state that Bielschowsky's method stains non-nervous components of tissue as well as nerves. Nonidez ('36, '37) reminds us that although some of the silver nitrate impregnation methods, such as Cajal's, do not stain connective tissue, Bielschowsky's method and its modifications do. Because much reliance was placed by the present writer upon tracing the course and continuities of a questionable structure, the advantages of the method of supravital staining with methylene blue, in which the entire section was mounted upon one slide, becomes evident, even though the axons often were not dyed as distinctly as when the Schultze-Stöhr silver technique was used. Whether or not a particular nerve was of the myelinated or the non-myelinated variety could be more easily distinguished in the specimen prepared according to the Schultze-Stöhr technique. In other words, in the supravital method of methylene blue there was loss of detail but greater ease in tracing. It should be understood that the strict criteria of nerve-tissue diagnosis were not applied in every instance when nerve fibers were recognized. Often the nervous nature of a fiber could be definitely told, even in the absence of Schwann's nuclei, by its tortuosity, by the presence of varicosities, by its staining properties and by its relation to another similar fiber. But strict criteria were applied in identifying questionable structures in tissues in which no typical nerves were detected hitherto. The author believes that in the ''squeezed'' preparations in which whole sections are mounted upon one slide it is impossible for stained fine nerve fibers to be present without being detected, since it should be easy to trace them to a definite nerve. Therefore, the probability of missing a stained nerve element by too rigid diagnostic requirements is virtually non-existent as far as this particular problem is concerned. This is especially true when one is reminded that large nerve trunks were always found in the abdominal umbilical vessels of both species. Furthermore, it must be added that no special diagnostic difficulties were encountered in the study of the umbilical cord proper, because no components of this structure, although well stained, ever bore a close resemblance to nerve tissue. That is to say, no varicosed fibers were found, although beaded threads were seen occasionally.

There still remains the probability of failure to stain the nerve elements in the cord proper, while the abdominal portions of the umbilical vessels show the affinity for the dye. This exigency cannot be positively excluded. Being mindful of the fact, brought out especially by the Russian histologists, that the very same nerve tissue, located in various parts of the same anatomical system, requires different staining time (cf. Schabadash regarding the staining of Auerbach's and Meissner's plexus in the intestinal tract), the author exposed the umbilical cord to a period of staining which fluctuated considerably from what was learned by experience to be the optimum time for the abdominal umbilical vessels. The results were overstained, or understained preparations, not fit for study. But by doing this, the time element as a possible factor in the production of negative results was excluded.

The finely beaded and varicosed network seen in the capillaries of the human fetal membranes and in the nutrient vessels of the guinea pig cord is an interesting and puzzling observation. Morphologically, the network considerably resembled fine nerve fibers. This resemblance brings to mind the illustrations of the nervous "terminal reticulum," shown in the publications of Stöhr, Jr. ('38) and others. The inability to show the continuity of this network with nerve fibers, or to find Schwann's cells makes its nervous origin very problematic. It could be argued that, in case of the human fetal membranes, the portion studied was selected at random and might have been nowhere near a large nerve fiber or trunk. But in the case of the guinea pig, where the entire cord with all its abdominal relations was mounted in toto, the connection with a large nerve branch should have been found had it existed. Although the resemblance of this network to nervous tissue is considerable, the author refrains from designating it as such, because of her inability to find connections between it and a known nerve fiber, the only acceptable criterion here. That a delicate network present in the walls of blood vessels of various organs might be of connective tissue origin is maintained by Nonidez ('36, '37), who was able many times to trace such a network to a collagenous fiber. He obtained his results by the silver carbonate method of Rio Hortega, and interpreted the delicate network as argyrophil (precollagenous) fibers.

Busch ('29) in his "Studies on the nerves of the blood vessels" shows a drawing of capillary "nerves" from the amnion of the guinea pig. He told of using whole preparations which were stained supravitally with methylene blue. According to him the doubtful structures could in many instances be traced to definite nerves. It would be interesting to know whether he traced the "nerves" of the particular preparation to an unmistakable nerve, and if he did, where did the nerve come from?

An attempt has been made in recent literature to correlate the newest anatomical findings of nerve distribution in the vessel wall with the accepted modus operandi of muscle stimulations. The paucity of nerve endings in comparison to the number of muscle cells, innervated by them has always been the stumbling block in the understanding of impulse conduction. The humoral theory, throwing light on such conduction, was developed on accumulated evidence that stimulation of nerve endings is accompanied by liberation of chemical substances which transmit the final impulse to the muscle cell. According to Stöhr, the finding of the widespread ''terminal reticulum'' fits in well with the humoral theory (provided, of course, that the nervous origin of the ''terminal reticulum'' is finally and definitely proven). That author looks upon the ''terminal reticulum'' or upon the Schwann cells which are intimately connected with it, as the possible anatomical substratum that liberates the excitation products. But even the newest conception of the transmission of nerve stimulation to the muscle cell does not throw light upon the mechanism of contraction of the umbilical blood vessels within the cord itself, which thus far have not definitely revealed a nerve supply. The humoral theory can hardly be applied to a structure which is as long as the umbilical cord and which is definitely supplied with nerves only in a small fraction of the length of its vessels.

The strongly developed musculature of the umbilical blood vessels in man is capable of powerful contractions, as is known through clinical observations and by experimentation. Strong contractions at the site of irritation were produced in man and animals by electrical, thermal and mechanical stimuli (Bucura, '02, '07: Rech, '25, and others). Unless the myogenic theory of blood vessel contractions can be accepted at the present time, the old query of Kölliker how to reconcile the contractility of the umbilical vessels with an apparent lack of nerve supply is pertinent even today.

SUMMARY

Modern neuro-histological methods appear to show that the umbilical blood vessels of the cord proper in man and guinea pig are devoid of a nervous apparatus.

The abdominal portions of the umbilical vessels in both species are richly supplied with non-myelinated nerve trunks

and nerve fibers, which in some areas form larger or smaller meshes.

It is significant that although both portions of the umbilical vessels (external and internal) were stained simultaneously as one preparation (guinea pig), nerves were discernible in the abdominal part only, for the umbilical cord proper gave negative findings.

Nerves of the abdominal umbilical blood vessels of the guinea pig were not detected at the navel or close to it. If that stretch possesses nerves, it was not revealed by the stain.

A network, found in the capillaries of fetal membranes in man, and in the nutrient vessels of the umbilical cord of the guinea pig, might suggest the nervous "terminal reticulum" of European authors. To the present author this network has not yielded definite proof of its nervous origin. That it belongs to connective tissue is perhaps more likely.

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BOOK REVIEW

ON GROWTH AND FORM. By D'Arcy Wentworth Thompson. A new edition. Cambridge and New York, University Press and Macmillan, 1942, 1116 pp., 2 plates, 554 illustrations, 21½ cm. Price, $12.50.

This unique book was first published in 1917. Now, after an interval of 25 years, the second edition, eagerly awaited by those familiar with the first, has appeared. The scope of the book and the general approach to the problems dealt with have remained unchanged, but considerable additions have been made and large parts have been recast. The new edition is conspicuously improved by a number of beautiful page-size pictures, for example, Edgerton's instantaneous photographs of a splash of milk (frontispiece), the paper-nautilus (p. 823), and the giant sun-flower (p. 913). On the other hand, many of the old pictures which had been perfectly clear in the first edition are almost valueless now because of crude offset reproduction (see, e.g., pp. 416, 749 and 844). Indications of the scale of the pictures are missing throughout.

The extent to which the author has kept abreast of recent developments in the experimental sciences is remarkable. Viruses, giant molecules, monolayers, x-ray-diffraction patterns of fibers, growth-controlling hormones, and the results of microdissection are mentioned in appropriate places in the new edition. On the other hand, it might have been wiser to avoid theoretical subjects like the theory of quanta (pp. 20 and 1094) and the uncertainty principle of Heisenberg (p. 123). Organic evolution and the second law of thermodynamics appear analogous to Thompson since both express a tendency toward the more probable states (pp. 11 and 358). One might as well consider both principles as opposite because organic evolution increases and entropy decreases organization. Entropy is, in any case, not the literal translation of evolution into Greek (p. 11).

A more elaborate presentation of mathematical procedures belongs with the assets of the new edition. Amplifications are found in Chapter III on "Rate of Growth," which is now twice as long as before. But here the new material has not been well organized. There is a subchapter "On variability, and on the curve of frequency or of error" (p. 118), and again another subchapter on "The curve of error" (p. 131). The factors on which the symmetry or skewness of a frequency curve may depend are mentioned in scattered remarks, but not summarized in a comprehensive way. On page 135 it is properly stated that ''the bell-shaped and the S-shaped curve form a reciprocal pair, the integral and the differential of one another,'' and in some figures this relation is well illustrated, e.g., figure 31. Other figures are lettered carelessly. For instance, the legend of figure 25 A is ''curve of frequency,'' while that of figure 25 B is ''curve of distribution,'' which is, of course, the same. In reality, figure 25 A shows the bell-shaped curve of increments, while figure 25 B gives the S-shaped cumulative curve of the same population of minnows. In Chapter VIII the new tables (pp. 597 and 598) make it much clearer how, in an aggregate of eight cells, the arrangement of their boundaries can be treated as a permutation problem; the two pages on topological analysis are another improvement. After the discussion of radiolarian skeletons in Chapter IX ''A note on polyhedra'' has been added. It embodies the findings that F. T. Lewis presented in this journal in 1933, but without reference to his paper. ''The broad, general principle'' (p. 737) which is particularly important in relation to cells, and which Thompson now recognizes, was clearly presented in that study; Prof. W. C. Graustein immediately saw that it was deducible from Euler's famous equation.[1]

The title of the book is ''On Growth and Form'' because form is the direct consequence of growth, whose ''varying rate in one direction or another has produced . . . the successive stages of development and the final configuration of the whole material structure'' (p. 57). The development to which Thompson refers is, however, neither phylogeny nor embryology; it is ''mathematical evolution'' in the sense in which the circle ''evolves,'' through innumerable ellipses, from the straight line (p. 869). Thompson rightly objects to having pictures of transition interpreted as if they were an evidence of actual sequence in time. Therefore one of the purposes of the book is to substitute mathematical relationships for hypothetical phylogenetic relationships. An example may illustrate this attempt. The outline of a human skull is drawn in Cartesian coordinates. These coordinates are transformed in such a curvilinear and non-equidistant projection that a chimpanzee's skull can be fitted into them. Finally, another projection is made to fit the skull of a baboon. The result is that the anatomical landmarks of the three skulls are located in corresponding places (with the same ordinal numbers) of the three different projections. In this way the degree of relationship of animals is established on the basis of ''topological similitude'' (Chapter XVII). Thompson criticizes the anthropologist's method of using axes and angles as frames of reference; ''in the anthropoid skull no rectilinear axis is homologous with a rectilinear axis in a man's skull, but what is a straight line in the one has become a certain definite curve in the other'' (pp. 1084–1085). It seems to the reviewer that Thompson's method of transformations can be very useful whenever configurations of several characteristics are to be described and compared.

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[1] Lewis, F. T. 1933 Mathematically precise features of epithelial mosaics. Anat. Rec., vol. 55, pp. 323–341. Professor Graustein's comment is found in Lewis, 1933, Proc. Amer. Acad. Arts and Sci., vol. 68, p. 264.

There is, on the other hand, a certain mysticism in Thompson's love for mathematics (cf. Epilogue p. 1097), a mysticism which induces him to take a mathematical description as an explanation, no matter what level of physical analysis the biological object has reached.[2] Then, mathematics becomes an obstacle rather than a help. For instance, in his discussion of the growth of antlers of deer (Chapter XIII) he is satisfied with what one may call mathematical models. He warns us not to attempt ''to inquire into the physical meaning or origin of this phenomenon.'' We should rest content with ''the main lesson'' that there is ''a complete contrast of mathematical properties between the horn and the antler'' (p. 896). These statements prove to be very misleading if one considers (1) the intermediary conditions between antlers and horns encountered in the pronghorn antelope (Antilocapra americana) and (2) Rhumbler's investigations ('29, not mentioned by Thompson) which show the similar influence of gravity on the form of both horn and antlers; obviously these two structures have important physical and biological properties in common.

The mathematical approach of Thompson is particularly successful when applied to those structures in which the logarithmic spiral is the common principle, namely, the shells of the nautilus, of snails, and of foraminifera. Since these structures grow by accretion, the writer of this review would suggest comparing them with crystals. Thompson states that ''crystals lie outside the province of this book,'' although snow-crystals are discussed in detail, mainly because of their beauty (p. 696). Instead of the numerous remarks about crystals, which are scattered throughout the book, a systematic treatment in a particular chapter would have been preferable. This would also give room for more consideration of F. M. Jaeger's excellent ''Lectures on the principle of symmetry and its applications in all natural sciences'' ('20), to which Thompson refers in a footnote only. On the whole Thompson's discussions of symmetry and asymmetry are comparatively weak; contradictory definitions of dextral and sinistral are used on page 819 and page 881.

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[2] In Aristotelian terms one may say that Thompson gives preference to the formal causes (''the ratio 2:1 is the cause of the octave'') over both efficient and final causes.

Thompson believes that biology can gradually be transformed into an exact science, although he does not expect physics and chemistry to account for all phenomena of life. He is also careful to state that his hope for an exact biology does not include the relations between body and soul (p. 13). To the writer of this review such a separation of body and soul appears more useful than any of the crude attempts to fuse biology with psychology. In biology, then, Thompson considers morphology as the last bulwark of irrational views (p. 1028 etc.). Therefore he tries to introduce mathematical treatment and physical views into morphology wherever it is possible: (1) by expressing the actual structures of animals and plants in mathematical terms as mentioned above; (2) by comparing them to the products of human engineering, which is mainly done in Chapter XVI; and (3) by means of analogies between organic structures and physico-chemical phenomena.

Any physico-chemical phenomena used for such an analogy will, according to general usage, be called ''models'' in the present review, although Thompson seems to dislike this term. There was a clear statement on the application and the limitations of artificial models in the first edition (p. 415), but unfortunately the best part of it is omitted in the new edition (p. 649). The most important question is, of course, how to apply a particular model to the given biological case. Let us take the analogy between the calycles of various species of Campanularia and different phases of splashes of liquids (Chapter V, p. 390). Some calycles are fluted with a notched margin, others have a notched margin without being fluted, while a third type is not fluted and has a smooth margin (p. 391). If each of these three forms corresponds to another phase of a splash, as Thompson assumes, he should tell us how to find out why each of the three types solidifies in another splash-phase. As the rate of formation is of quite different orders of magnitude in a splash of milk and a calycle, what properties should a model-liquid have in order to produce a slow development of lasting forms? Are clay and glass the proper material (p. 392)? Strangely enough, Thompson rarely makes any suggestions which would encourage actual experimentation.

Yet Thompson hopes that his work will be a step toward making biology an exact science. It is generally agreed that an exact science is characterized both by careful observations and quantitative treatment of the data obtained. In Thompson's book one realizes a tendency to over-emphasize mathematical and physical deductions at the expense of well-established observations. In Chapter II ''On magnitude'' he explains the physical reasons why only very small animals, like humming birds and insects, are capable of stationary flight, while birds of pigeon-size and larger are not. Unfortunately he overlooked the kestrel which, although larger than a pigeon, is famous for hovering. In Chapter XIII a new paragraph deals with the asymmetry of the skull in dolphins. Thompson states that the action of the tail of the dolphin is similar to that of a screw-propeller, and that the "counter-spirality of the skull is the direct result of the spiral component in locomotion." It has escaped Thompson's notice that a similar theory of "unequal water-pressure" had been advanced by Kükenthal in 1908. The two arguments which abolished Kükenthal's thesis also invalidate Thompson's theory, viz., (1) all whales swim probably in a similar way, but only some groups have a definite asymmetry of the skull; (2) in those species which have a conspicuous asymmetry of the skull the asymmetry is already pronounced in the embryo.

The strangest example of this type is Thompson's analysis of those physical forces in the oviduct of the hen which cause the "streamlining" of the egg (Chapter XV). The whole discussion is based on the erroneous assumption that, because the blunt end is usually laid first,[3] it also travels foremost in the oviduct. In 1918 Bartelmez published the interesting history of this puzzle and its eventual solution by Wickmann. Moreover, Thompson's mistake has been pointed out by Needham in his "Chemical Embryology" in 1931.

One of the greatest paradoxes of the book is Thompson's hostile attitude toward genetics (pp. 340–341). Why does he speak of "the mysterious field of force in which the chromosomes lie," when he professes to care much more for the configuration of forces than for their nature? If he had really confined himself to "the simpler outward phenomena of organic growth and structure," as he states in his program (p. 14), one could understand his lack of appreciation of cytogenetics. But he devotes the whole of Chapter IV to the "internal formal structure of the cell," inclusive of the arrangement of chromosomes. Genetics being based on the high correlation between cytological findings and statistical numbers should appeal to Thompson, although other biologists may be disappointed that, as yet, genetics has not been able to bridge the ontogenetic gap between chromosomes and phenotypically visible characteristics.

It is impossible to express a summarizing opinion of Thompson's book. It is a peculiar mixture of scientific and amateurish procedures. "Growth and Form" is certainly not a reference book in the ordinary sense, although surprising things can be found in it. The table of contents is much too short, and the index is inadequate. The subproblems of growth and form are not indexed at all; one can hardly guess that the item "growing point" is discussed in a chapter called

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[3] Thompson says that this was known to Albertus Magnus, but it is already mentioned in Aristotle's "De generatione animalium."

''The form of tissues.'' Wrong page-references are numerous, evidently due to lack of assistance in wartime.

Is it a stimulating book? The best criterion for answering this question would be the number of investigations which were actually inspired by the first edition. F. T. Lewis states explicitly that it was ''Growth and Form'' which led him to his investigations on the shape of cells. In this case Thompson had asked the precise question whether cells of vegetable parenchyma are dodecahedra or tetrakaidecahedra, and Lewis could decide in favor of the second alternative. That was an unexpected result. So was the further finding of J. W. Marvin (Columbia University) that compressed shot are also 14-hedral. Moreover, E. B. Matzke observes that a froth of soap-bubbles, of as nearly uniform size as they can be made, does not exhibit the Kelvin 14-hedral pattern. Such foam displays an array of irregular cell-like shapes, accountable quite readily once they have been observed. Thompson's presentation of this matter in Chapter VII of the new edition does not show to what extent the clearly demonstrated results of Lewis and his school (as Sinnott and Bloch have recently put it) are contrary to a priori mathematical expectation. Evidently, the most difficult but at the same time most important task is to link mathematical consideration with actual investigations. In most cases Thompson's brilliant ideas are framed in such a general way that few investigators will take the trouble to transform them into operational problems. The main value of the book lies in the fact that it is a fascinating guide for traveling an unusual route through the worlds of aesthetics, mathematics, mechanical engineering, colloidal chemistry, biology, and the history of science.

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THE BLOOD VESSELS OF HUMAN CELIAC GANGLIA AND CHANGES IN THEIR VASCULAR PATTERN ASSOCIATED WITH AGE

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ONE TEXT FIGURE AND TWO PLATES (SEVENTEEN FIGURES)

It has been shown by previous investigations (Bergmann and Alexander, '41; Bergmann, '42) that the vascular bed of sensory ganglia in man exhibits certain peculiarities not encountered in other parts of the nervous system. The question arises whether the dilatations and ampullae found in capillaries and small vessels of sensory ganglia of persons beyond the second or third decade of life are characteristics of sensory ganglia, or whether other parts of the nervous system of comparable structure share the vascular pattern with cerebrospinal ganglia. The celiac ganglion of man was chosen to answer this question. Gross and microscopic features of the vessels were studied. Emphasis was laid on the vascular pattern in the ganglia themselves, since details of the gross arterial supply have been worked out by D'Evant ('01), Delamare and Tanasesco ('06) and Machado de Souza ('32). Cytological features are not included in the present publication except as casual observations. Detailed discussions of the cytology are found in the literature (De Castro, '32; Kuntz, '38 a). Pathological investigations of the ganglia have been made by Staemmler ('23), Terplan ('26) and more recently by Kuntz ('38 b). Adams ('42) reviews the problem of the vascular supply of nerves and presents a most complete bibliography.

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MATERIAL AND METHODS

The celiac ganglia of forty-four human bodies which came to autopsy at the Boston City Hospital were used. Their ages ranged from that of premature infants to 83 years. The ganglia of forty-one cases (usually the ganglion of one side only) were fixed in formaldehyde solution 1: water 9 parts, cut as frozen sections 300 micra thick, and stained according to a modified Lepehne-Pickworth technique (Doherty, Suh and Alexander, '38). In these specimens the vascular pattern could be readily visualized in three dimensions. In order to study the gross supply of the celiac plexus a method which did not interfere with the routine post mortem technique had to be

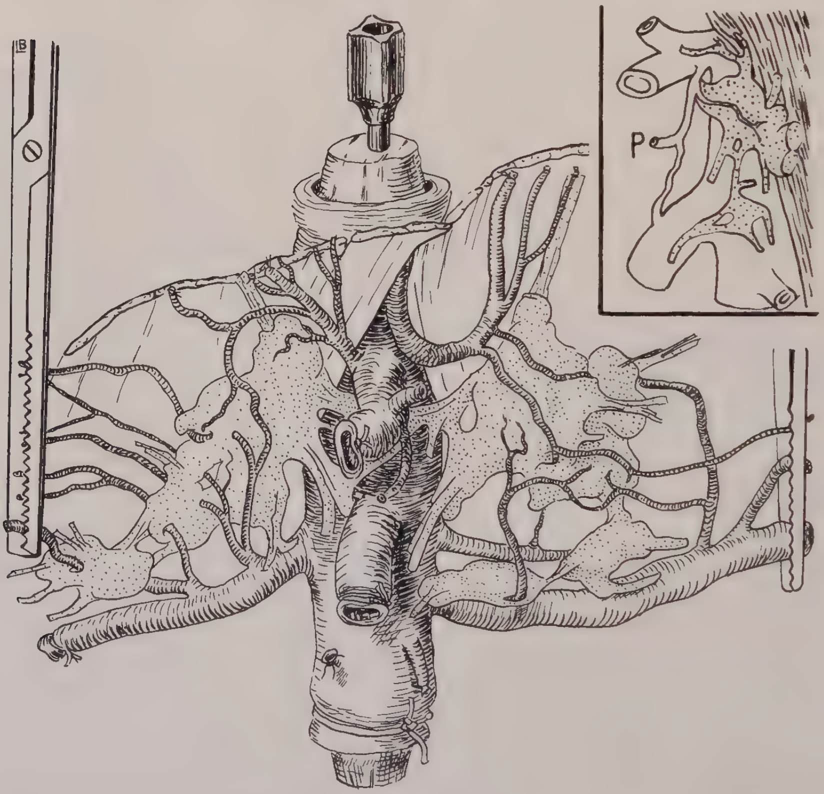

Fig. 1 Injection specimen spread out, showing the gross arterial supply of a celiac ganglion. P, branch to pancreas. Explanation of figure and inset in the text.

used. In three instances the celiac ganglia were removed in situ with a large block of surrounding tissue and injected with a mixture of India ink and formaldehyde from the aorta. Leakage during injection was stopped as far as possible by hemostats and ligatures. Figure 1 shows the extent of the block and the method of injection. The three injected cases were not used for histological studies and therefore do not appear in the table. The blood vessels of several other cases were dissected under a lens without injection, before histological preparations were made.

To obtain information on histological details, ganglia of cases representing various age groups were fixed in formaldehyde or Zenker's fluid. Frozen sections stained with hematoxylin-phloxine or paraffin sections 3 or 4 micra thick were prepared. The paraffin sections were stained with hematoxylin-phloxine, Regaud's hematoxylin, Van Gieson, Masson's trichrome stain, Mallory's aniline blue, Cresylviolet and Verhoeff's elastic tissue stain. The reticulum was impregnated with silver according to Foot's method on Zenker material or according to Gömöri's or Wilder's method on formaldehyde-fixed tissue. Occasionally Eichorn's ('41) "zenkerizing" technique for formaldehyde-fixed tissue was employed.

OBSERVATIONS

The arteries. The authors who examined the arterial supply of the celiac ganglia (D'Evant, '01; Delamare and Tanasesco, '06; and Machado de Souza, '32) recorded sufficient details, but failed to emphasize the unusually high variability of the arterial sources. No two ganglia show an identical arterial supply. This is in conformity with the marked variations in shape, size and extent found in the ganglia themselves (Robinson, '07; Kiss and Ballon, '29; Hovelacque, '27). Figure 1 shows the arrangement found in a 52-year-old female body. The arteries are small and spring mainly from the inferior diaphragmatic, renal and suprarenal vessels, from lumbar arteries and from the frequent anastomoses which connect these vessels. Many of these vessels actually perforate the ganglion itself. In addition one often finds small vascular twigs accompanying some of the nerve bundles to the ganglion; the most constant vessel being an artery running with the splanchnic nerve. This branch is derived from intercostal arteries and is joined by diaphragmatic vessels.

The celiac ganglia share their arterial supply to a large extent with the suprarenal glands. The variability of the arteries in this region has been recognized by Gérard ('13), Anson, Pick and Beaton ('39) in man, and in the cat by Bennett and Kilham ('40). However, while Gérard ('13) and Anson, Pick and Beaton ('39) found adrenal arteries springing from intestinal branches of the aorta in man, I was not able to find branches of the celiac or mesenteric arteries supplying the celiac ganglia. In the cat several adrenal branches derived from intestinal arteries were described by Bennett and Kilham ('40). Similar branches participate in supplying the celiac ganglia of the cat (Konikov and Bergmann, to be published).

A branch to the left celiac ganglion springing from an anastomosis between the stems of the celiac and superior mesenteric arteries is pictured in the inset of figure 1. This anastomosis constitutes a remnant of the embryonic "ventral longitudinal anastomosis" between the segmental roots of the omphalomesenteric artery (Tandler, '04). It has been noted here because of its rarity and embryological significance.

The small size and variability of the nutrient vessels to the celiac ganglia makes it questionable whether the exact course of the arteries in any particular case is of any importance. It seems sufficient to emphasize that the celiac ganglia are supplied by the parietal arteries of their environment.

The arteries of supply give off small branches which run in the connective tissue capsule of the ganglion or in fibrous strands within the ganglion. The channels which enter the actual nervous tissue of the celiac plexus are very small, even if derived from a larger extraneous vessel. They divide at acute angles without visible precapillary anastomoses (fig. 3). Arterio-venous anastomoses as described by Nonidez ('42) in sympathetic chain ganglia of the dog were not seen in human celiac ganglia.

The walls of the arteries in the older age groups are often thicker than in young individuals, and many show an increase in fibromuscular tissue in the media or hyalinization of the wall. The lumen is sometimes narrowed (fig. 8), sometimes almost to the point of obliteration.

The veins. The venous channels draining the celiac ganglia go to the adrenal and diaphragmatic veins and pass through the fine plexus of veins which lies in the extraneous connective tissue around the ganglia. The veins of the ganglion proper do not intercommunicate freely or form any sort of plexus, thus differing from the veins of the extraneous connective tissue. There are a few anastomoses between the veins of the nervous tissue and those of the surrounding connective tissue, which in turn, contains veins passing into the mesentery and the area behind the pancreas. Thus the possibility exists for venous drainage from the celiac ganglia into the portal system.

The small vessels. A section through a celiac ganglion shows three types of tissue grouped in a most irregular and varying fashion: First collagenous connective tissue derived from the capsule and from the sheaths of vessels and nerve bundles entering the ganglion; second, bundles of nerve fibers; and third, aggregates of ganglion cells. Each of these tissues is characterized by a specific vascular pattern (fig. 5). While the connective tissue possesses blood vessels comparable to the vascular architecture of any other fibrous masses, the nerve bundles are supplied by widely scattered small vessels which form long, slender capillary loops. The ganglion cell complexes are abundantly supplied by a dense capillary system. Dunning and Wolff ('37) drew the interesting conclusion from their findings in the cat, that the vascularity is highest in regions occupied by terminations and synapses of neurons.

Within the cell complexes of infants the capillaries form a dense and regular network of even caliber. The vessels supplying and draining the capillaries are of only slightly larger diameter, a feature contributing to an impression of a uniform vascular bed (fig. 2).

In the first decade of life the difference between the diameters of capillaries and larger channels increases and all elements of the vascular bed, except the arteries, begin to show a certain degree of tortuosity.

After the second decade of life the blood vessels not only show a well developed differentiation into large and small channels, but also exhibit dilatations which may extend over a considerable stretch (fig. 3) or even form round or pear-shaped ampullae (figs. 4 and 6). The larger dilatations (30 to 100 micra in diameter) occur in the small venules and pre-capillaries, while true capillary ampullae rarely measure as much as 30 micra in diameter. As shown in the table, such dilatations and ampullae were found to some degree in all cases from the third decade of life onward. In this respect the celiac ganglia show clearly the same vascular peculiarities as do human sensory ganglia.

Definitive ampullae and dilatations appear only in the vessels amongst nerve cells. The very largest dilatations with diameters of 80 and more micra are usually found near the borders of ganglion cell aggregates, sometimes actually a slight distance outside the clump, amongst neighboring fibers (fig. 9). The walls of these very large dilatations are thin, but differ from those of true capillaries in that they are composed of layers of fibrous tissue. The presence of elastic tissue is questionable. Treated according to Verhoeff's method some fibers take a faint stain unlike the intense black of the elastic fibers in arteries in the same section. Hyalinization is a frequent finding in the walls of these vessels (fig. 9).

Tortuous vessels appear in the ganglia in the second decade, though they are also seen occasionally in younger specimens. They are most conspicuous amongst the ganglion cells, but are entirely lacking in the connective tissue (fig. 5).

In the eighth decade of life the vascular bed of the celiac ganglion becomes less abundant, and ampullae and dilatations

TABLE 1

CASE NO.	AGE	SEX	VASCULAR PATTERN	DIAGNOSIS
1	47 days	m	r	Prematurity
2	1 hour	f	r	Multiple anomalies
3	9 hours	m	r	Birth trauma
4	3 days	f	r	Subdural hematoma, pneumonia
5	16 months	m	r	Waterhouse-Friderichsen syndrome
6	6 years	m	r t	Asphyxia, whooping cough, mongoloid
7	11 years	m	r(t)	Pistol shot through head
8	14 years	m	(t)	Rheumatic heart disease
9	17 years	m	t d	Rheumatic heart disease
10	21 years	f	d (a)	Tuberculosis, pyelonephritis
11	33 years	f	t d (a)	Meningeoma (sarcoma)
12	33 years	f	d (a)	Rheumatic heart disease
13	36 years	f	t d (a)	Tb. pulm., congenital heart disease
14	37 years	f	(t) d (a)	Rheumatic heart disease, pneumococcal meningitis
15	38 years	f	d	Miliary tuberculosis, pneumonia
16	38 years	m	t d a	Rheumatic heart disease
17	45 years	m	t d a	Cerebral hemorrhage, hypertensive heart disease
18	49 years	f	t d a	Rheumatic heart disease, cerebral infarct
19	50 years	m	t d (a)	Hypertensive heart disease, pneumonia
20	50 years	m	d a	Uremia, pyelonephritis
21	51 years	f	t d a	Hypertensive heart disease, pyelonephritis
22	52 years	m	t d a	Alcoholism, heart disease
23	54 years	m	t d a	Arteriosclerosis, encephalomalacia
24	55 years	m	t d a	Aortic stenosis, arteriosclerosis
25	56 years	f	d (a)	Lues cerebrospinalis, cerebral thrombosis
26	60 years	f	d a	Alcoholic cirrhosis, arteriosclerosis
27	61 years	f	t d a	Coronary disease, arteriosclerosis
28	63 years	m	t d a	Pneumonia, septicemia, hypertensive heart disease
29	63 years	f	t d a	Cerebral infarct, hypertensive heart disease
30	67 years	m	t d a	Prostate hypertrophy, pyelonephritis
31	68 years	m	t d a	Hypertensive heart disease
32	69 years	m	t d a	Branchiogenic malignancy, old tb., atheromatosis
33	70 years	m	d a	Bronchopneumonia, prostate hypertrophy
34	70 years	m	d a	Diabetes mellitus, gangrene of leg, arteriosclerosis
35	72 years	f	d (a)	Cerebral infarct, hypertensive heart disease
36	76 years	f	d a	Diabetes mellitus, hypertensive heart disease
37	76 years	f	t d a	Coronary disease
38	79 years	f	d a	Pulmonary embolism, cancer of stomach, arteriosclerosis
39	80 years	m	(d)(a)	Pneumonia, arteriosclerosis
40	82 years	f	d a	Nephrosclerosis, atheromatosis, pneumonia
41	83 years	f	(d)(a)	Arteriosclerosis

The vascular pattern is described by: r — regular network; t — tortuosities; d — dilated stretches of vessels; a — ampullar dilatations. Parentheses are used to indicate those cases where the respective feature does not constitute a conspicuous finding.

are not as conspicuous as in the younger adult cases. This involution of the vascular bed accompanies the increase in connective tissue in the older age groups described by Staemmler ('23), Terplan ('26) and Kuntz ('38 b). The first two of these authors even speak of a "sclerosis" in reference to an increase in fibrous tissue in sympathetic ganglia.

The relationships of the vessels to the ganglion cells. The capillaries of celiac ganglia of infants are closely applied to the reticulum capsule of the ganglion cells. However, each individual cell is not supplied with its own capillary, since frequently a group of neurons is lodged in a common capsule of reticulum which in turn is surrounded but not invaded by vessels. The cells within this capsule may or may not be separated by a single delicate layer of argyrophil reticular fibers (figs. 15 and 18). The reticulum of the capillaries is loosely woven (fig. 18), and may simulate the reticular sheath enclosing a neuronal process.

In ganglia of cases beyond infancy the individual neurons within a cluster are separated from each other by a considerable amount of reticulum or collagenous tissue and by capillaries. Thus almost every neuron is adjacent to one or more capillaries, and is separated from them by the fused reticular layers of the wall of the vessel and of the cell capsule. With advancing age the pericapillary reticulum becomes denser in texture (figs. 16 and 17).

The capillary ampullae also lie close to the ganglion cells and they frequently indent the capsule of the neuron or even the ganglion cell itself, displacing the satellite cell corona at the point of contact (fig. 10). Some groups of ampullae appear to compress the neighboring neurons or at least to occupy all available space between them (figs. 7 and 13). Impregnation with silver shows that the neurons are always separated from the vessels by some reticulum fibers which are derived from the capillary wall as well as from the cell capsule (fig. 11). As has been mentioned before, the very largest ampullae and dilatations may be slightly distant from the ganglion cells.

The appearance of the neurons themselves has been described by authors concerned with the normal and pathological histology of celiac ganglia. In the present studies also one finds a great variety of cells, ranging from normal to highly degenerate forms. Neither degenerative changes nor pigmentation could be correlated with age or underlying pathology. Binucleated cells are occasionally seen in adults (fig. 12). Since they neither possess a constriction nor are composed of two cells lodged in a common reticular capsule, they do not conform to De Castro's ('32) criteria of late normal division. They probably represent abnormal cells, though one can not count them among the degenerative forms. In infants, binucleated ganglion cells are more numerous, (figs. 14 and 15), and generally regarded as delayed stages of normal cleavage. In adults, Terplan ('26) frequently found neurons with two nuclei. Spielmeyer ('22), reviewing experimental data believes such cells to be neurons which have incompletely divided under pathological stimuli. An interesting bibliography covering this subject can be found in a more recent article by Beaton, Holmes and Windle ('39).

DISCUSSION

Although sympathetic and sensory ganglia have been the frequent object of critical morphological investigations, the peculiar capillary ampullae and dilatations have not been previously recorded. In former studies carried out on thin sections, such ampullar dilatations were apparently interpreted as an "increased capillary bed" (Gardner, '40), or hyperemia. Ganglia which were examined as a whole after injection and clearing (Machado de Souza, '32) had been removed from newborn infants and therefore ampullae were not seen. In the present material the apparently inconspicuous vascular bed in a thin paraffin section was in sharp contrast with the abundant pattern seen in the thick benzidine slide, where the vessels could be followed for considerable distances disclosing their ampullae in a three-dimensional picture.

The possibility that the dilated capillaries are due to acute engorgement can not be entertained, since the vascular bed of the immediate neighborhood of the ganglia, namely the vessels in the surrounding connective tissue, do not take part in the formation of locally dilated channels. The wide capillary ampullae suggest rather a replacement of neurons which undergo degeneration, as outlined by Bergmann and Alexander ('41). Degenerating neurons are met frequently in the celiac ganglia; the question whether the ampullae compress the cells or simply take the place of cells which have undergone degeneration can not be answered. Since ampullar dilatations are found close to cells in all transitions between the normal state and extreme degeneration (fig. 7), one might surmise that the capillaries dilate primarily or independently and do not fill in the space where neurons have degenerated.

Hypertensive and arteriosclerotic cases form the vast majority of the older individuals examined. No attempt is made in this publication to correlate the alterations in the vascular bed of celiac ganglia to theories which either advocate or preclude the sympathetic system as a factor in the development of hypertension. Cantor ('42) who measured the weights of celiac ganglia definitely denied a relation between his figures and hypertension. Terplan ('26) examined the neurons and the interstitial tissue on a large series of adult cases and was reluctant to make specific statements as to the significance of structural changes in sympathetic ganglia. Kuntz ('38 b) arrived at the logical conclusion that changes within a given age group should only be considered characteristic for this group if uniformly present, while occasional variations met in several groups could be due to morbid processes.

In evaluating the findings presented in this paper, one has to keep in mind that it is hardly possible to collect material from normal individuals representing all age groups. Therefore, the material can not be looked upon as a true cross section of the normal postnatal development of the vascular bed of the ganglia. However, the ampullae and dilatations are typical for celiac ganglia of all persons from the third decade of life onward.

SUMMARY

Human celiac ganglia from autopsies were studied in order to determine the vascular anatomy and the relationships of the blood vessels to the ganglion cells. The following findings were noted:

1. The gross arterial supply is derived in variable fashion from small branches springing primarily from the diaphragmatic, renal, suprarenal and lumbar arteries. The veins drain into corresponding channels and anastomose with dense networks in the extraneous areolar tissue.

2. Ampullae and dilatations of capillaries and small vessels within the ganglion, similar to those in sensory ganglia, occur in all cases beyond the second decade of life. These alterations of the vascular pattern have no apparent relation to the underlying pathology and are absent in the young; they are considered a manifestation of age. The ampullae lie in regions occupied by nerve cells and are separated from the neurons in most instances merely by reticulum derived from the capillary wall and the capsule of the cell. Tortuous vessels are often present. Vessels of the connective tissue stroma show neither ampullae nor tortuosities.

3. The perivascular reticulum fibrils become denser and coarser with age.

4. Pathological changes in the walls of some vessels are described.

ACKNOWLEDGMENT

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PLATE 1

EXPLANATION OF FIGURES

2 Frozen section stained with benzidine. Celiac ganglion of a premature infant (case 1) showing the regular vascular network characteristic of infants. A large vein shows in the upper left corner; a nerve bundle almost without vessels is at the extreme right. 70 ×.

3 Frozen section, benzidine stain. A celiac ganglion of a 36-year-old woman (case 13) showing characteristic dilatations in the vessels of a cell cluster. Note the numerous tortuous vessels; a small straight artery at the bottom center. 60 ×.

4 Benzidine stain of a frozen section through a ganglion of a 63-year-old woman (case 29) showing tortuous vessels, ampullae and dilatations. 65 ×.

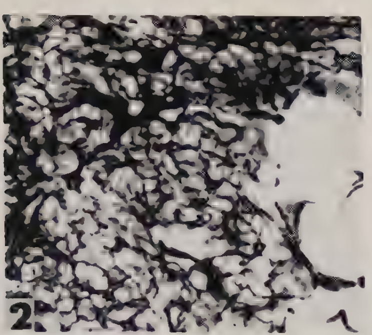
5 Benzidine stain of a frozen section through a ganglion of a 68-year-old man (case 31). The characteristic vascular pattern in each of the three types of tissue found in celiac ganglia is shown. To the left is a nerve bundle; in the center, extraneous connective tissue; to the right, ganglion cell aggregates. Note that only the vessels among cells show ampullae and dilatations. 23 ×.

6 Frozen section, benzidine stain; 82-year-old woman (case 40). Large ampullae are seen in a group of nerve cells, traversed by a strand of nerve fibers. 55 ×.

7 Paraffin section stained with Regaud's hematoxylin. Celiac ganglion of a 68-year-old man (case 31). This section shows the appearance of ampullae in an ordinary paraffin slide. Some ganglion cells are vacuolated. 300 ×.

8 Frozen section stained with hematoxylin-phloxine. Ganglion of a 60-year-old woman (case 26), showing thickening of the media of an arteriole with narrowed lumen. 650 ×.

9 Frozen section stained with hematoxylin-phloxine. Celiac ganglion of a 63-year-old woman (case 29) showing a very large dilatation of a venule with hyalinized wall. 150 ×.



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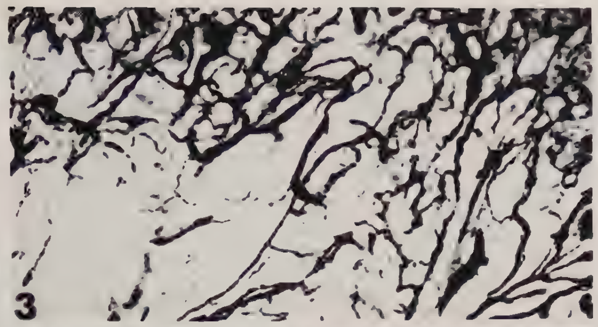

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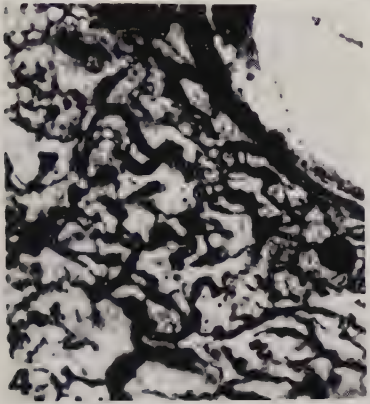

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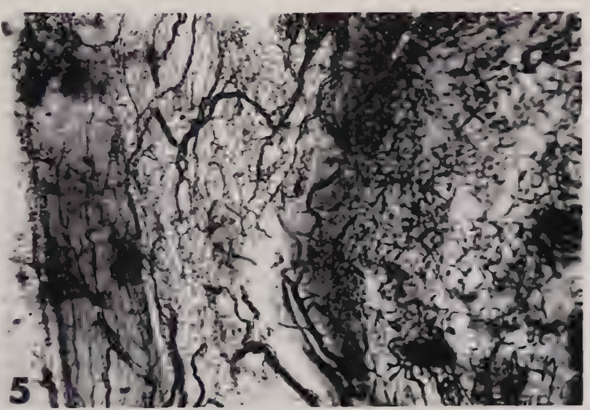

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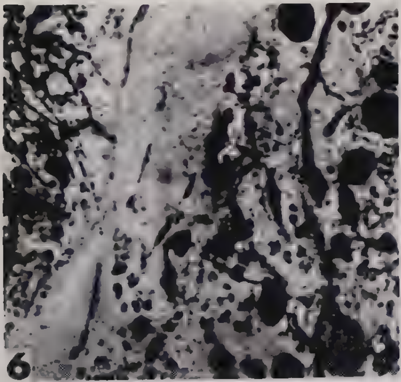

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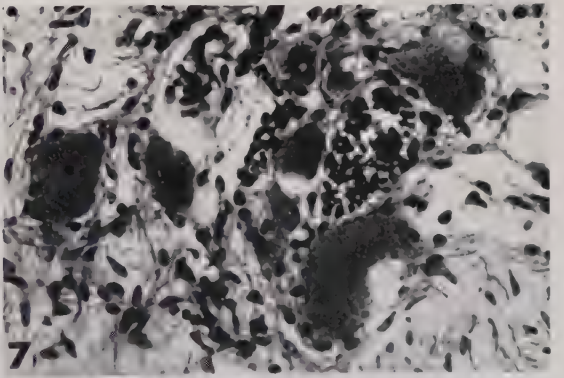

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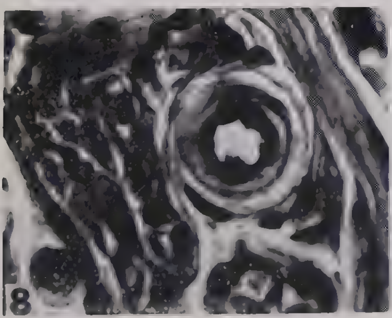

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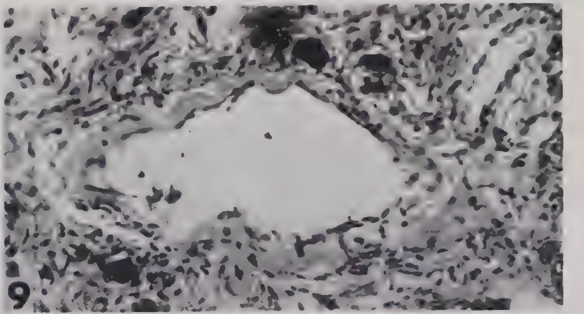

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PLATE 2

EXPLANATION OF FIGURES

10 Paraffin section stained with hematoxylin-phloxine. Celiac ganglion of an 82-year-old woman (case 40). A capillary ampulla is apparently directly adjacent to a neuron which seems to be indented by the vessel. 650 ×.

11 Same section as shown in figure 10, after decolorization. Impregnation of the reticulum according to Gömöri. The capsule of the neuron is fused with the pericapillary reticulum. 650 ×.

12 Paraffin section, hematoxylin-phloxine. From a ganglion of a 17-year-old boy (case 9). A binucleated ganglion cell rather isolated and not, as often seen in infants, in a cluster. 540 ×.

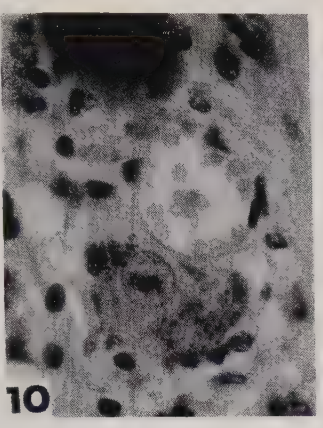
13 Paraffin section, impregnated with silver according to Wilder. Celiac ganglion of a 55-year-old man (case 24) showing the general arrangement of the reticulum between ampullae and groups of nerve cells. A vein is at the lower left corner.

14 Paraffin section, impregnated with silver according to Gömöri. Celiac ganglion of a newborn infant (case 2). The reticular sheath of a neuronal process is visible extending downwards. It is continuous with the capsule of the binucleated ganglion cell. 610 ×.

15 Another field of the same slide as shown in figure 14, showing a cell cluster surrounded by well developed reticulum; the partitions between the individual cells are delicate and do not contain capillaries. Note a binucleated neuron at the top of the cluster. 610 ×.

16 and 17 Paraffin section impregnated with silver according to Foot. Celiac ganglion of a 56-year-old woman (case 25). In figure 16 the reticular sheath of a neuronal process is brought into focus. It is more delicately woven than the framework of the capillary wall which is brought into focus in figure 17. Note in the right part of figure 16 a capsule of a cell cut tangentially; at the top of figure 17 an ampullar widening of the capillary. 750 ×.

18 Paraffin section impregnated with silver according to Gömöri. Celiac ganglion of a newborn infant (case 2). The loosely woven reticulum meshes of a capillary are shown, contrasting to the vascular wall shown in figure 17. 1150 ×.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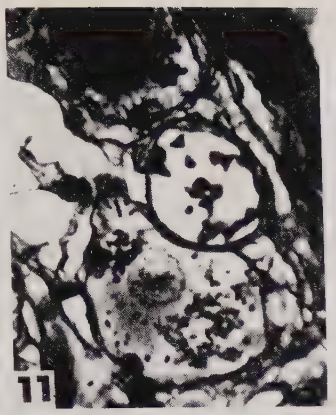
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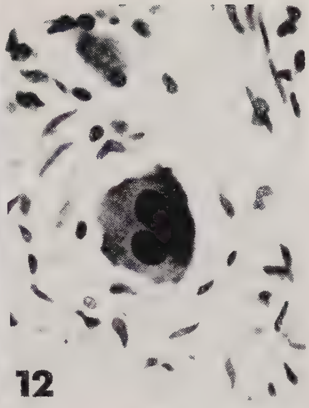
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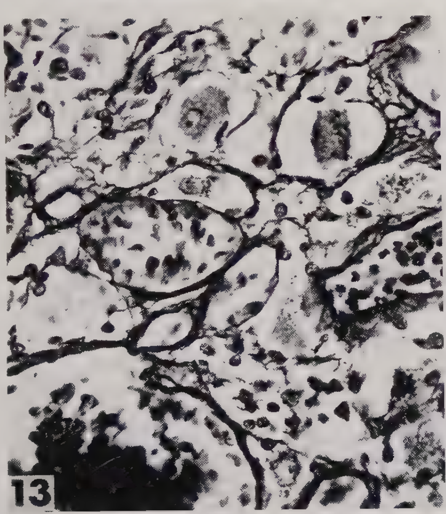
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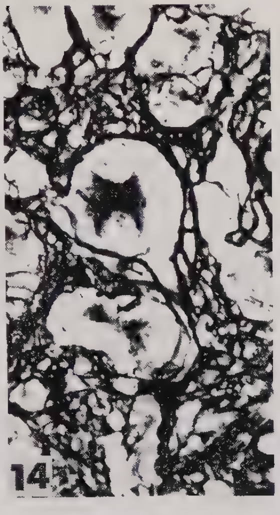
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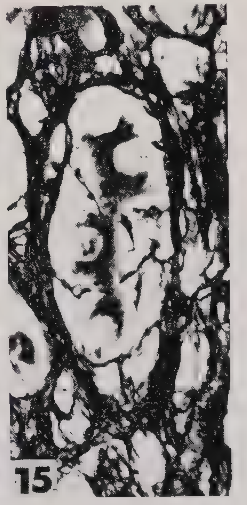
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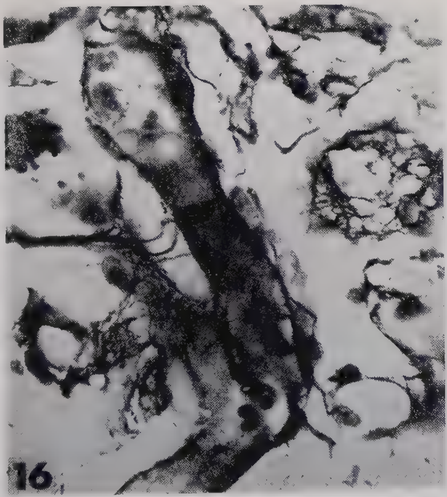
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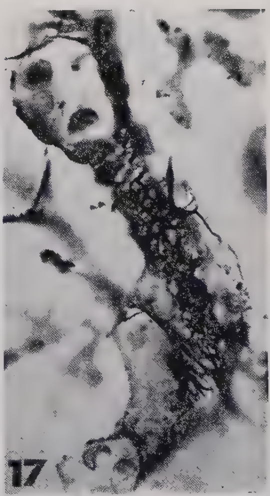
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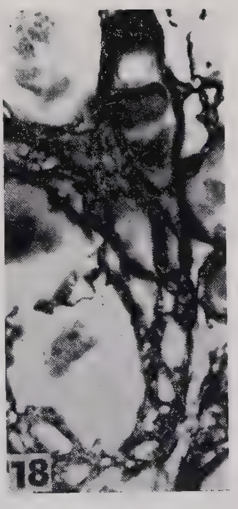
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A STUDY OF CERTAIN CELLS OBSERVED IN THE GALL-BLADDER MUCOSA OF CATS

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THREE PLATES (SEVEN FIGURES)

The epithelium of the gall-bladder is usually considered to be a simple columnar epithelium and as such is briefly described in text books of histology. Probably because of this, relatively few investigators have concerned themselves with detailed histological descriptions of the epithelium lining this organ. One of the earliest of these was given by Virchow in 1857 and is still considered to be a classical description of the gall-bladder epithelium. In his observations on man (adult and children), dogs and cats, he describes the epithelium in some detail and reports it very similar to the structure of intestinal epithelium. He discusses the presence of fat granules in some of the cells and believes this fat to be in the process of absorption. Aside from the careful descriptions of the usual tall columnar cells and the cells filled with fat, the writer makes no mention of any other cell type. Kuhn (1883) gives a careful analysis of the histological structure of the bile ducts and gall-bladders in some of the larger domestic animals. The mention he makes of the epithelium is limited to a careful description of the usual columnar cell type. Rudinger (1895) describes the gall-bladder of a decapitated man. He mentions the penetration of the mucosal surface by leucocytic cells which push between the columnar cells of the epithelium and can later be demonstrated in the bile, where they disintegrate and contribute their substance to the bile. He fails, however, to mention any granular material present in the cells such as that found by the writer in the cells of the cat. Aschoff ('06) discusses fat absorption in the gall-bladder mucosa of dogs. A more extensive paper dealing with the histology of the gall-bladder is the one by Jurisch published in 1909. He deplores the lack of detailed histological work on the gall-bladder and calls attention to the divergent opinions concerning the finer cytological changes in the epithelium, i.e., cuticula, fat infiltration and secretion (mucous). His observations were made on adult humans of various ages (both normal and pathological), human embryos (6 months) and newborn infants. He likewise studied gall-bladders from a number of animals: dogs, cats (mature and newborn), goats, lambs, pigs, calves and oxen, guinea pigs and rabbits. Jurisch made a very careful study of the cells of the epithelial layer in the investigated animals and describes in detail the cytological changes in these cells. He reports finding goblet cells in all species examined except rabbits and guinea pigs and notes variations in shape of the usual columnar cells — very slender cells appearing interspersed among the cells of the epithelial layer. He carefully describes the fat infiltrated cells and states that he failed to find fat droplets in epithelial cells of rabbits, guinea pigs and newborn children, although fat was demonstrated in the other layers of the wall of the gall-bladder. In dogs, cats and goats he reports a strong fat infiltration in the epithelial cells of varying quantities, depending on the time of last feeding of the animals. This paper is one of the most careful and detailed studies of the gall-bladder epithelium that had been published up to that time, moreover, it gives fixing fluids, stains, etc. which most of the early German writers fail to do. In spite of this careful analysis of the cells, the author fails to mention cells of the type which are described later in this study.

Descriptions dealing with gall-bladder epithelium to be found in standard works on comparative histology are likewise meagre and are based, for the most part, on the idea that the epithelium is of a simple columnar variety. Probably one of the best known works on comparative histology is Oppel's work entitled ''Lehrbuch der vergleichenden mikroskopischen Anatomie'' published in 1900. In describing the epithelium in mammals (pigs, sheep, guinea pigs, dogs and man) the author speaks very briefly of tall narrow columnar cells and mentions cells containing fat droplets. One of the more recent detailed descriptions of the epithelium lining the gall-bladder of the cat is by Winkenwerder ('30) who gives a careful description of the cells. First, he reported two morphologically different types of cells in the mucosa of the gall-bladder, but later decided that these were merely different functional stages of the same cell, basing this assumption on the presence of a further transitional type of cell which he observed. He found the two extreme cell types in the proportion of one ''slender cell'' to fifteen ''columnar cells.'' (The slender cells described by Winkenwerder were known to the older writers and have been described by Sudler, '01; Aschoff, '06; Jurisch, '09 and others.) Boyden ('26) gives the following description of the mucosa in cats, ''The mucosa of the gall-bladder and of the accessory gall-bladders consists of a simple columnar epithelium thrown into rugose folds, the summits of which, as seen in cross section . . . . appear to be distended with loose tunica propria through which runs a coarse capillary network . . . . the mechanism for the absorption and concentration of bile.''

Much of the work on the physiology of the gall-bladder has dealt with mechanical factors in gall-bladder function and with chemical analysis of the bile before and after various experimental procedures. Relatively few investigators have made any attempt to correlate their findings with careful histological study of the mucosa.

Several studies (Auster and Crohn, '22; Mann, '24; Greene, Bercovitz and Hanssen, '35) indicate that so far as secretion is concerned, it is probably true that under normal conditions a small quantity of mucoid secretion, probably nucleo albumen (Bollman, '40), is produced by the gall-bladder proper. Reports of Drury ('24), Riegel, Ravdin and Johnston ('32) and Harding ('34) indicate that the secretion of large quantities of mucus occurs only in response to damage or irritation of the mucosa. Some authorities concede that small quantities of cholesterol and calcium are also secreted under normal conditions by the mucosal cells. Elman and Taussig ('31) concluded from their work on dogs that cholesterol is secreted by the gall-bladder mucosa, and presented evidence that the quantity of cholesterol secreted may be increased by infection. One of the difficulties encountered in such studies is to determine whether the cholesterol is being secreted or absorbed. Wilkie and Doubilet ('33) found that when the cystic duct of dogs was tied, cholesterol could pass in either direction, depending on the relative concentration of the cholesterol in the bile and blood. In this experiment, it appears that histological preparations were made to check the normalcy of the tissue, but no description is given of the cells of the mucosa. Their conclusions were reached by colorimetric determinations of cholesterol in blood and bile. With reference to the secretion of calcium, Johnson, Ravdin, Austin and Morrison ('31–'32) conclude from their work on dogs that small amounts of calcium are secreted into the gall-bladder only when it is damaged. On the other hand, Andrews, Dostal and Hrdina ('33) state that calcium is excreted in large amounts by the mucosa of the normal gall-bladder and they consider this an important factor in the formation of gallstones. There are indications that possibly the hormone secretin may be secreted by the gall-bladder mucosa (Johnson, '22), and according to Mann ('24) exogenous substances such as Rose Bengal may be excreted by these cells.

It is an established fact that the gall-bladder is capable of concentrating the hepatic bile which enters it. From experimental evidence (Billard and Cavalié, '00; Fox, '27; Sweet, '29; Rous and McMaster, '21; McMaster and Elman, '25; Ravdin, et al., '32), it appears that the normal gall-bladder is able to concentrate the hepatic bile from six to ten times. Most of this concentration is due to the absorption of water. In regard to the absorptive activity of the gall-bladder, Harding ('34) writes, "A large amount of fluid is absorbed by the gall-bladder from the bile; with this there is also an absorption of salts, mainly of sodium chloride but also some calcium salt, some absorption of cholesterol and bile salts in relatively fixed proportions occurs, but not in great amounts. There is some evidence for the absorption of other fats, but it is not proven that this happens regularly. Bile pigment is probably absorbed, but only in very small amounts.'' A detailed and critical discussion of the absorptive activity of the gall-bladder is given by Ivy ('34). Since the publication of his review, a number of articles on gall-bladder absorption have appeared in the literature. In 1935 Rousselot and Bauman reported that cholesterol was absorbed from the gall-bladder. Their study included histological examinations of gall-bladder sections stained with H & E and Scharlach R. Lloyd ('40) studying the comparative concentration of hepatic bilirubin and cholesterol by the human gall-bladder on post mortem material, found evidence ''that in certain instances cholesterol may be either added to or removed from the gall-bladder''. Riegel, Calder and Ravdin ('40) using more refined techniques verified their former findings that cholesterol is not absorbed from hepatic bile placed in the normal bile-free gall-bladder. Johnson, Ellis and Riegel ('37) in experiments on dogs to test absorption of sodium tetraiodophenolphthalein, found that the dye is absorbed more rapidly in the damaged gall-bladder than in the normal organ.

In none of the literature was there reference to cells like those observed by the author while examining the gall-bladders of cats. In order to check the constancy with which these unfamiliar cells occurred, the gall-bladders of fifty-nine cats were examined. Of this number, fourteen were discarded because of autolysis of the mucosa; the findings on the remaining forty-five cats are presented in this paper.

MATERIALS AND METHODS

This paper reports the findings on forty-five mature cats, twenty-four of which had undergone operation for the interruption of the supraoptico-hypophyseal tract, a procedure not related to the purposes of the present investigation. In some of these animals the suprarenal glands had also been removed. Five of the animals had been subjected to adrenalectomy alone. The remaining sixteen animals were normal.

In forty-four of the cats the gall-bladders were removed immediately after death, the bile drained and pieces of the tissue placed in various fixatives. Since the granular cells were first observed in material fixed in Helly's fluid and in formalin (5 to 10% solutions), sections of each gall-bladder were placed in either one or the other, or both, as a control. Other sections of these same gall-bladders were placed in various other fixatives including Bouin's, absolute alcohol, Champy's and osmic acid. In one instance, an animal was anesthetized and then perfused with 10% formalin in saline. After perfusion was complete, the gall-bladder was removed and a portion of it was placed in 10% formalin and the remainder was placed in Helly's fixative. All of the material was embedded in paraffin and serial sections cut at 8 micra were stained with Harris' hematoxylin and eosin, Mallory's connective tissue stain, Heidenhain's iron hematoxylin, Hoyer's thionin and several special stains which will be discussed later in the paper.

OBSERVATIONS

The epithelium of the gall-bladder of the cat, for the most part, consists of tall columnar cells with the nuclei located in the basal half of the cell. The width of these cells varies considerably, ranging from cells whose widths are about half that of their length, to those whose widths are one-fifth or less of the cell length, the so-called "slender cells" of Winkenwerder ('30), who has given adequate and comprehensive discussion of the columnar cells of the gall-bladder mucosa of the cat. Among the columnar cells, scattered cells characterized by large, bright pink granules (H & E stain) of variable size in the cytoplasm were observed. For descriptive purposes these cells will be referred to throughout the remainder of this paper as "granular cells".

The granular cells were found in the epithelial layer, wedged between the columnar cells (fig. 1). Most of these cells were located in the lower part of the epithelium. Only in occasional cases did they extend high enough into the epithelium to reach the lumen of the bladder. In a few instances, however, they were observed apparently discharging the granular contents of the cells into the lumen. In many places the cells were below the row of columnar cell nuclei, but careful examination showed them to be superficial to the underlying connective tissue core rather than within it.

The cells varied in size, apparently according to the quantity of granular material present in their cytoplasm. In shape they were round or oval. Occasionally an elongated, sausage-shaped one, which stretched the entire width of the epithelial layer appeared; these distorted configurations were possibly due to pressure.

The cell membrane was distinct in the smaller cells, but as the granular mass increased and the cells became larger, the membrane became thinner and more difficult to discern. Its presence in some instances could only be assumed by the retention of the shape of the granular mass. The granules themselves were characteristic, possessing distinctive staining properties which made them conspicuous against the clear homogeneous cytoplasm of the cells. With Heidenhain's iron-hematoxylin the granules stained black; Harris' hematoxylin and eosin showed them as bright pink staining masses, and they took a similar pink color with Mallory's connective tissue stain. Their size was extremely variable. In the majority of the cells they were about the size of the granules in an eosinophilic leucocyte. However, in many they were larger, the size varying within the same cell (figs. 2, 5 and 6). This variability in size within a cell suggested that there might be a coalescence of some of the smaller granules. In a few cells the cytoplasm contained only one or two large granules (fig. 4, a). In such instances the center of the granule stained very lightly while around the margin the color was typical of the smaller granular masses.

The cells in which the granules occurred possessed round or oval nuclei. In those containing small granules, the nuclei appeared to be centrally placed and were similar in form and staining qualities to nuclei of small lymphocytes. In other instances they were peripherally placed, stained darkly and appeared small and rather triangular in shape, resembling the nuclei of goblet cells. This occasional peripheral position was presumably the result of displacement by pressure exerted by the granular mass. In a very few cells the nuclei assumed such peculiar shapes as can be seen in figure 3, a, where one is hammer-shaped. These unusual forms, however, occurred very infrequently.

Examination of numerous sections impressed the observer with several distinctive features. One of these impressions was that the granular cells were ''pushing up'' into the epithelium. This impression received support from the presence of large numbers of the cells in the lower part of the epithelium and located along the lower edge of the epithelial layer. Moreover, in those sections in which the number of granular cells was relatively insignificant, it was observed that there were many small lymphocytes in the subepithelial connective tissue. When the lymphocytes were present in large numbers, some of them showed certain characteristics which might be considered as transitional stages to an ultimate granular cell phase. In the vicinity of the epithelium some of the lymphocytes appeared to develop a clear area around their nuclei. This perinuclear area was regular in shape and appeared to be unstained cytoplasm. In cells becoming more intimately related to the epithelium, this clear cytoplasmic area became wider and more distinct. Cells of this sort could be found pushing up into the epithelium, forcing their way between the columnar cells. In size and shape, as well as in nuclear configuration and position, these elements appeared identical with the granular cells, differing from them only in the absence of the acidophilic granules in the cytoplasm. On all of the sections studied only a single cell of this type was found in which the cytoplasm contained very small faint pink staining granules. These granules could be seen only with high power and by careful focusing.

From these observations, the author suggests that these granular cells are derived from the lymphoid cells of the subepithelial tissue which have migrated into the epithelium of the gall-bladder.[1]

Quantitative data

The granular cells were found in the gall-bladder mucosae of all of the cats investigated, although the number of cells present in the different cats varied enormously. In order to estimate the number of cells, centimeter wide strips were cut from the middle of the gall-bladders of ten cats, prepared as previously indicated and serial sections were cut. Every tenth section was stained with Harris' hematoxylin and eosin and the granular cells in each stained section were counted. Averages were then computed for each cat. The average number of granular cells per cross section per cat was found to range from 1.5 cells in cat no. 45 (normal male), to 303 cells in cat no. 54 (normal female). In the majority of the cats, however, the average per cross section was less than 50.0 cells.

Any attempts to correlate quantity of granular cells present with previous operative procedures or sex was unsuccessful. Through the cooperation of Dr. A. C. Ivy of Northwestern University and Dr. D. G. Sattler of Cornell University material was made available from cats of those localities in an effort to determine whether these granular cells might result from some condition peculiar to the local animals. However, gall-bladders taken from all cats of the Chicago and New York areas showed the presence of these granular cells.

Comparative data

The question arose as to whether these cells were peculiar to cats or whether they were to be found in the gall-bladder mucosa of other animals. In order to answer this question partially, gall-bladders of dogs, rabbits, and frogs were examined. Material from eleven dogs, five white rabbits, and nine frogs was prepared in the manner previously described. All animals examined were normal adults. No attempt will be made at this time to describe the histology of the gall-bladder in these forms; it is sufficient to state that in none of the specimens examined did the author find granular cells such as were found in and described for the cat.

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[1] The author wishes to express her appreciation to Dr. William Bloom who, after checking and rechecking the material, confirmed the lymphoid nature of the granular cells.

DISCUSSION

The granular cells described above have occurred in appreciable numbers in all the specimens examined. It seems strange that they were not present in the preparations examined so carefully by Winkenwerder ('30). It might be that they are peculiar to cats of a certain locality, perhaps the result of some dietary or pathogenic influence. However, the finding of granular cells in the cats from Chicago and New York would seem to rule out this possibility. The significance of these cells is not known. They may be the expression of either secretory or absorptive activity of the gall-bladder. Unfortunately, from the preparations at present available one cannot be certain in which direction the material is going, whether from the cells into the lumen of the gall-bladder or from the lumen, through the mucosal cells, into either the lymphatic or blood streams. If secretory or absorptive, the type of activity must be peculiar to the cat and should be found in animals of all localities. On the other hand it is conceivable that they might be cellular inclusions due to a parasitic infection of the gall-bladder, or to some filtrable virus affecting the mucosal cells. Examination of these possibilities revealed no evidence in favor of their adoption. A number of specialists have examined the sections and declared the granules are not parasitic in nature, nor the result of virus infections.[2]

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[2] In seeking the opinion of an authority on diseases of small animals, Prof. H. C. Smith of the Department of Veterinary Medicine, Oklahoma Agricultural and Mechanical College, was consulted. After examining representative preparations, Professor Smith stated that in his opinion the granules were not inclusion bodies.

The possibility that these cells might be Russell body cells has been considered by the author. So-called Russell body cells are most often found in pathological lesions or in areas of recent pathology. They are found in relation to disintegrating plasma cells and their distribution is restricted to the connective tissues rather than epithelium (G. Dubreuil and M. Favre, '20; Fick, '08; Weill, '19; Cowdry, '28; et al.). Russell bodies are both intra- and intercellular. Since the cells reported by the author do not correspond with the above mentioned characteristics, the possibility of their being Russell body cells was discarded.

The morphological similarity of these granular cells to the "Schollenleukozyten" of Weill ('19) has been suggested. Weill found the Schollenleukozyten in the alimentary epithelium of the dog, cat, mouse, rabbit, and pig. He regarded the cells as having a lymphatic origin and considered the globules to be endogenous secretion granules and the cells comparable to the fuchsin-body cell or the atypical mast cell, although he failed to offer experimental explanation of the nature of the cells. Similar cells were also observed by Keasbey ('23) in the alimentary mucosa of sheep and cows and were described under the name of globule or plaque leucocytes. Through the use of special stains, Keasbey found the granules in the cells reacted in a manner identical with the red blood cells and in her conclusions states, "These globules although almost solely of epithelial occurrence appear to contain haemoglobin."

The striking similarity between Keasbey's description of the morphology of the "globule cells" and those which the author found in the mucosa of the gall-bladder of cats led in this study to the application of some of Keasbey's staining techniques to the gall-bladder material. Special stains used included tests for iron (both Nishimura's method and the modification of this method suggested by Keasbey); Graham's method for selective staining of granular leukocytes, which is based on the oxidation of benzidine by a peroxidase which the granules contain; and Okajima's elective stain for erythrocytes. With Nishimura's method and Keasbey's modification, the test for iron in the granules gave negative results although iron was nicely demonstrated in the red blood cells. Graham's benzidine stain likewise failed to give identical staining reactions for the two elements. The red blood cells gave a yellowish-brown or brownish coloration, while the granules remained unstained. They remained unstained also if a methylene blue counterstain was used, but stained a bright pink if eosin was used as the counterstain. Therefore, these granules did not contain a peroxidase such as is contained in hemoglobin. Only with Okajima's elective stain for erythrocytes did the red blood cells and the granules stain the same color (brown). Concerning the use of this stain, however, Keasbey states, "I do not regard this stain as a test for haemoglobin, in as much as Okajima rests his claim only upon empirical trial of his test on tissues and on haemoglobin; he offers no chemical explanation for his observation." Although the staining reactions between the granular cells and the "globule" cells of Keasbey were not identical, the morphological similarity between the two was so marked as to suggest a possible close relationship and it is conceivable that any differences found might be the result of difference in location of the two types of cells.

It is not within the scope of this particular paper to discuss the whole problem of these granules, however, certain characteristics of the granular cells should be reemphasized. They were present in all of the cat material examined. Helly's fixative proved to be the most satisfactory of any of the fixatives used. The granules showed an affinity for eosin in H & E and Mallory's stain, indicating their acidophilic nature. With Heidenhain's iron-hematoxylin they stained black as did the red blood cells. They did not appear to be lipoid since they were not soluble in ordinary fat solvents (alcohol, xylol, etc.) which would have removed them during the routine procedure of preparing the tissues. Moreover, they did not reduce osmic acid. In comparing their staining reactions with those of red blood cells, with Mallory's stain the red blood cells took a copper color while the granules stained the bright pink of eosin. Iron tests gave negative results. With Okajima's technique for elective staining of erythrocytes, both the red blood cells and the granules stained a reddish orange, but in the use of this stain attention should be called to Keasbey's criticism. The granules did not contain a peroxidase such as is found in red blood cells.

Incidental to the study of the cells described, some interesting peculiarities of the nuclei of certain columnar mucosal cells were observed. In some of these cells, the nuclei contained what appeared to be a globule of a clear homogeneous unstained material (fig. 3, b). This material, as judged by the size of the globules, varied in quantity in different cells and appeared to force the chromatin material toward the periphery of the nucleus. In the elongated nuclei, the chromatin mass was forced into the ends of the nuclei where it formed semi-lunar areas. It is possible to make out the nucleolus in one of the semi-lunar areas in figure 4, b. In other sections the globules appear at the distal end of the nucleus. The significance of this material in the nucleus is not known. It is conceivable that these "globules" represent a material produced by the nucleus. The possibility that they are nuclear inclusions must not be overlooked. On the other hand, we may be dealing with a simple artefact produced by fixation. The chief argument against this is the fact that various stages of this condition appear on the same section with normal nuclei, where the fixation treatment is identical for both.

CONCLUSIONS

1. A hitherto unreported "granular cell" was observed in the mucosa of the gall-bladder of the cat. The cells were round or oval in shape with the nuclei in some oval and centrally placed and in others pyramidal in shape and peripherally located. The cytoplasm of these cells contained a variable number of irregular sized granules which stained a bright pink with Harris' hematoxylin and eosin, and Mallory's connective tissue stain. With Heidenhain's iron-hematoxylin the gran-

ules stained black. They did not reduce osmic acid. When tested for iron, the results were negative. The granules did not contain a peroxidase such as is contained in hemoglobin. These cells were found, for the most part, wedged between the columnar cells of the epithelium. However, some were found between the subepithelial core and the epithelium, and occasionally a few were found extending through the epithelium to the lumen of the gall-bladder.

Further work is being carried out in an attempt to clarify the problems presented by the granular cells, and to determine, if possible, their relation to the functional activity of the gall-bladder.

2. Lymphocytes were found in the subepithelial connective tissue. In some instances, those nearest the epithelium appeared to have developed a clear zone of cytoplasm around the nucleus. Some of these cells were found pushing up between the columnar cells of the epithelium and appeared identical with the granular cells except for the lack of granules in the cytoplasm.

3. In the nuclei of a few of the columnar cells round globules of clear homogeneous material of varying sizes were observed. Sometimes this globule appeared in the center of the nucleus and seemed to force the chromatin material into the ends of the nucleus where it formed semi-lunar areas. In other cells the globules appeared at the end of the nuclei nearest the lumen of the gall-bladder. The nature of this globular material is unknown.

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PLATE 1

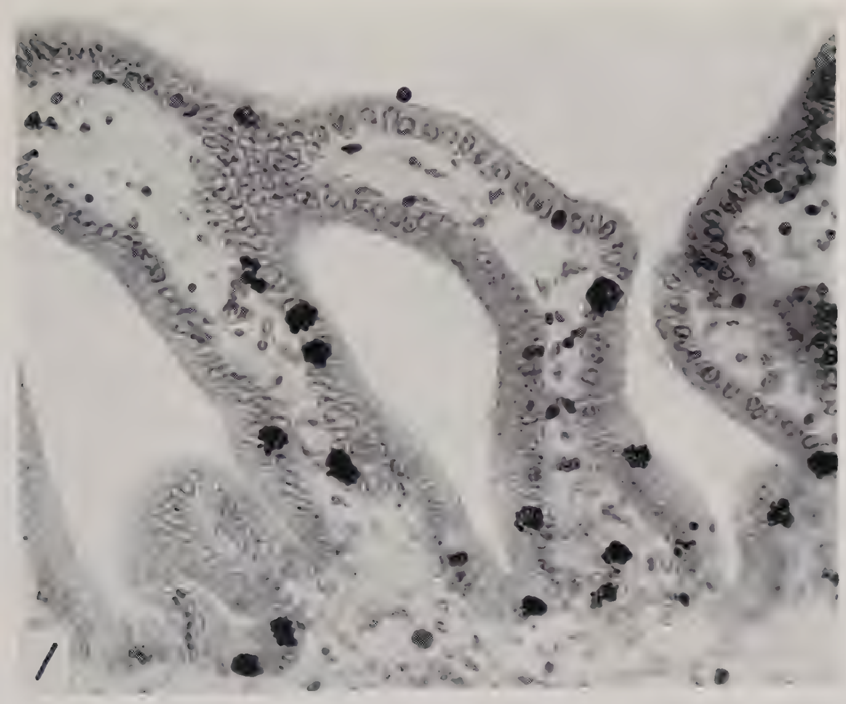
EXPLANATION OF FIGURES

The photomicrographs were taken by Dr. Kendrick Hare

All sections are of Zenker-formaldehyde fixed tissue. Sections in figures 1, 2, 5, and 7 are stained with Heidenhain's iron hematoxylin. The sections in figures 3, 4, and 6 are stained with Harris' hematoxylin and eosin.

1 Photomicrograph showing numerous granular cells (black areas) in the gall-bladder epithelium of a cat. × 300.

2 Photomicrograph. Granular cells in gall-bladder epithelium of a cat. Note variation in size of the granules. × 600.



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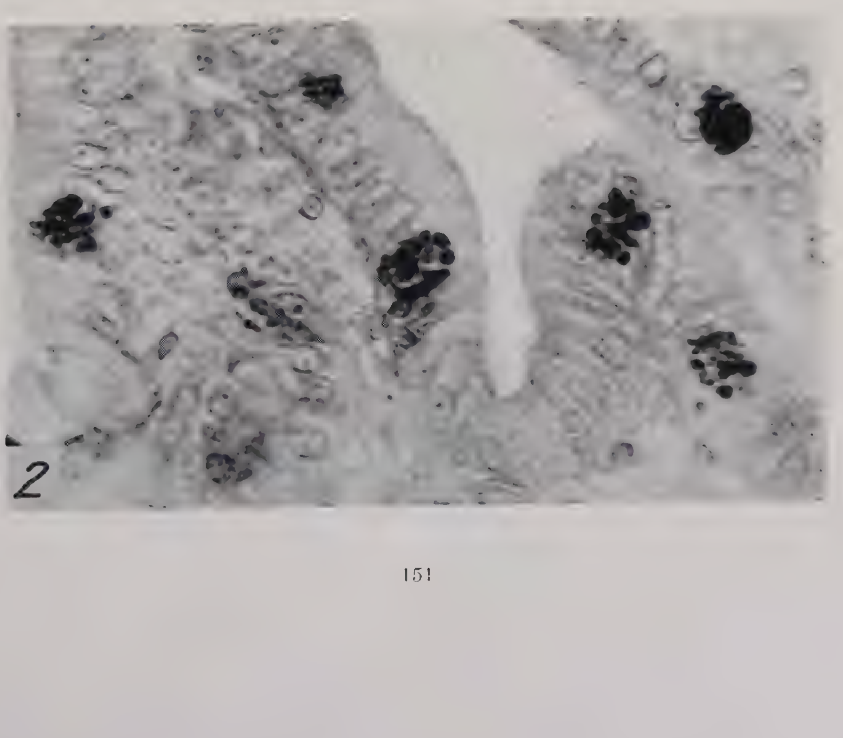


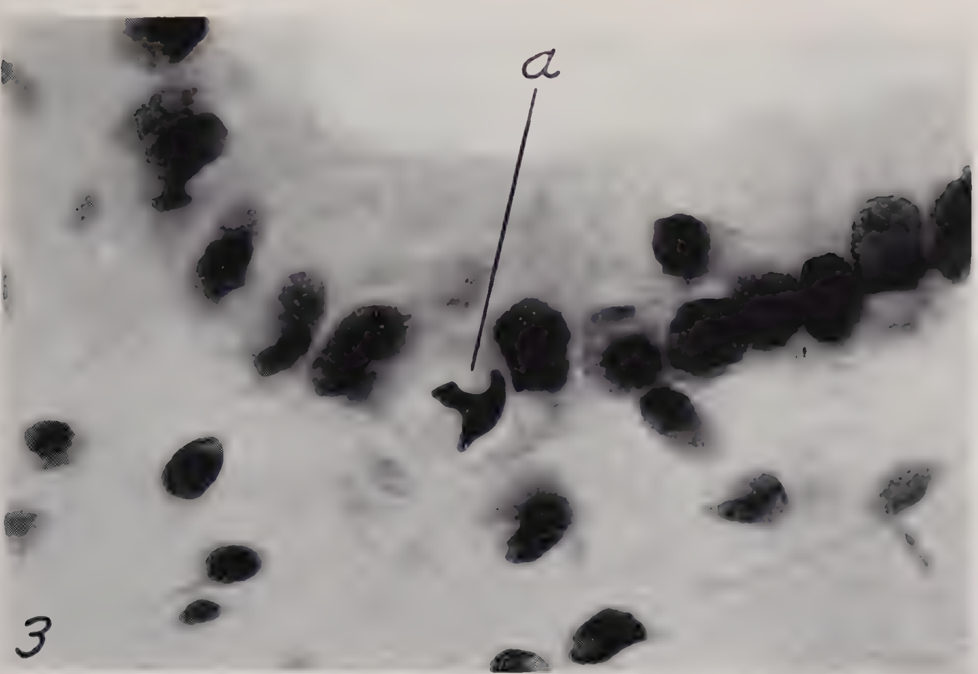
2

PLATE 2

EXPLANATION OF FIGURES

3 Photomicrograph showing gall-bladder epithelium of a cat. (a) A large round granular cell having a well defined cell membrane. The cytoplasm is filled with refractive red staining granules. The nucleus is hammer-shaped; the chromatic material is dense and darkly staining. (Photomicrograph has been retouched to bring out free border of epithelium). Oil immersion.

4 Photomicrograph. Gall-bladder epithelium of a cat showing (a) a granular cell containing only two large granules; (b) a tall columnar cell with globule of homogenous material in the nucleus. This material seems to force the chromatin material into crescentric shaped masses at either end of the nucleus. (The photomicrograph has been retouched to bring out the free border of the epithelium). Oil immersion.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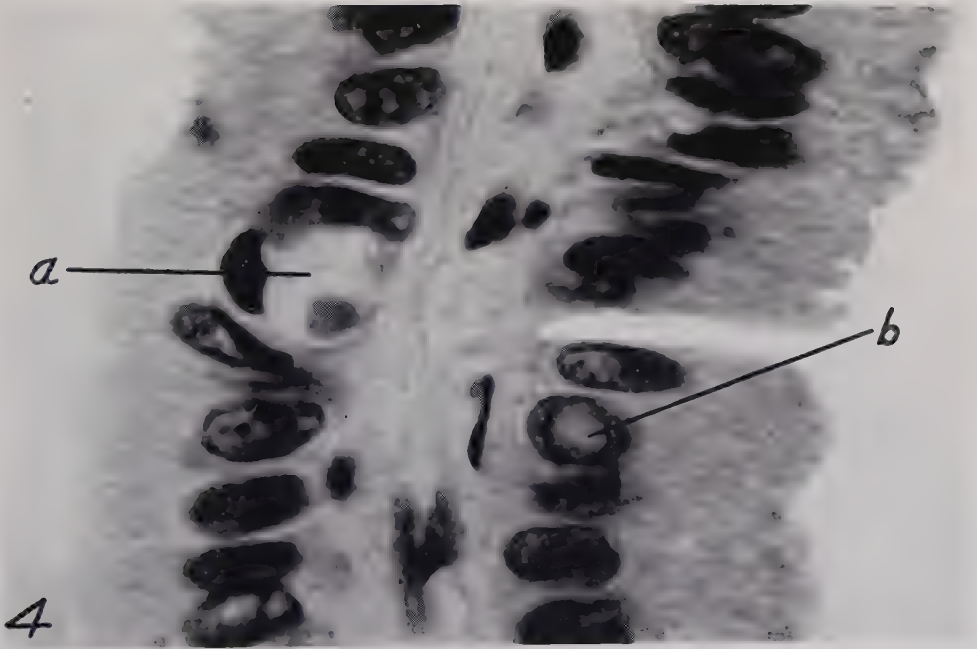
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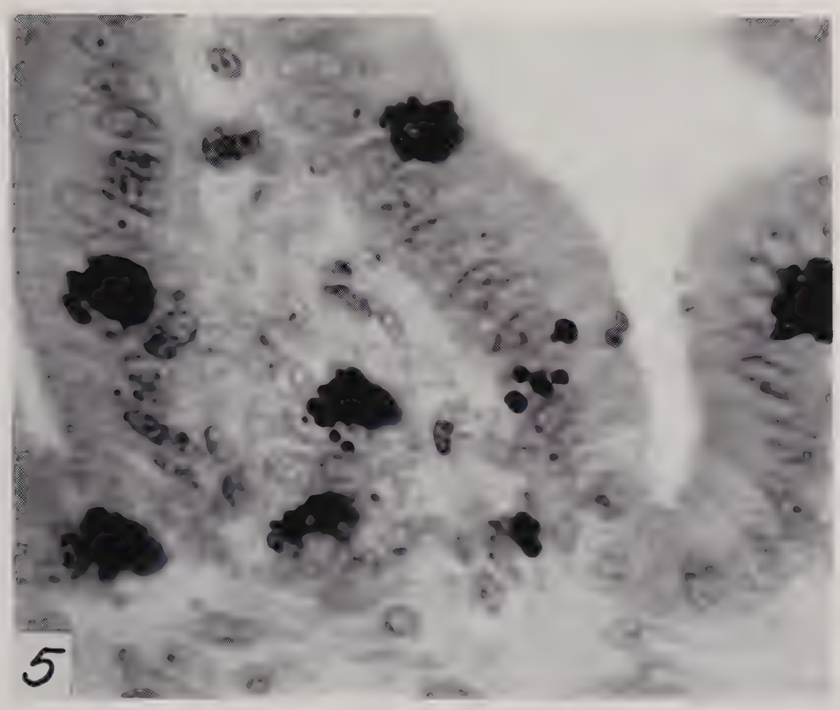
PLATE 3

EXPLANATION OF FIGURES

5 Photomicrograph. Gall-bladder epithelium of a cat showing variation in size of granules. × 750.

6 Photomicrograph. Gall-bladder epithelium of a cat. Note variation in size of the granules within a single cell and their refractive appearance. × 600.

7 Photomicrograph. Gall-bladder epithelium of a cat showing a granular cell in which the granules are small and regular in size. × 600.



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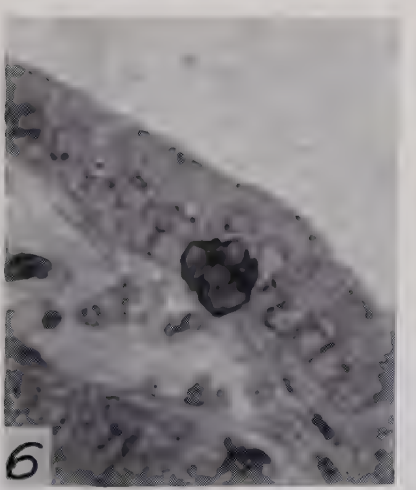

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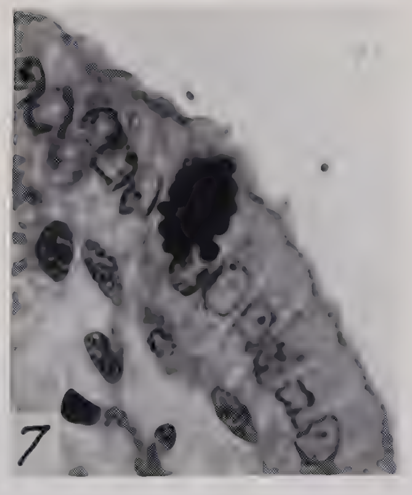

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REPORT OF AN EXCEPTIONAL CASE OF LINEAL ARTERIAL TORTUOSITY

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ONE FIGURE

INTRODUCTION

Extreme spiraling of the lienal artery merits comment even though the artery is noted for its tortuosity. This paper reports such a case.

During routine dissection in the gross anatomy laboratory in the fall of 1941, there was called to the attention of the staff the unusual appearance of the lienal artery herein described. On first inspection, dissection not being complete, the artery was only partially revealed, and had the appearance of a coiled spring situated on the anterosuperior aspect of the body of the pancreas with a slight regression of the coils toward the left. The singularity of the specimen was noted, and the dissection of the artery and surrounding structures was continued by the author. The remainder of the peritoneum, fascia, and connective tissue was carefully removed from the anterior aspect of the artery, and the artery was freed from the fascia attaching it to the body of the pancreas. In turn, the dissection of the pancreas, lienal vein, and celiac axis was completed. Drawings were made in situ of the artery and surrounding viscera (fig. 1). The spleen, pancreas, liver, the lienal artery, the trunk of the celiac axis, and associated structures were then removed. The parts removed were photographed and preserved.

LITERATURE

Few references to the degree or pattern of tortuosity in the lienal artery were found. Springorum ('33) furnishes an acceptable index for tortuosity: $\mathrm{I} = {}^{100\,\underline{\mathrm{A}}}$, in which L represents the Luftline, or airline distance between the origin of the artery and the point of its last division before it enters the hilus of the spleen, A is the actual length of the artery between these same two points, and multiplied by 100 to express the index in per cent. Therefore if the artery pursued a straight line, the index should be 100. The extreme indices noted by Springorum were 120 and 350.

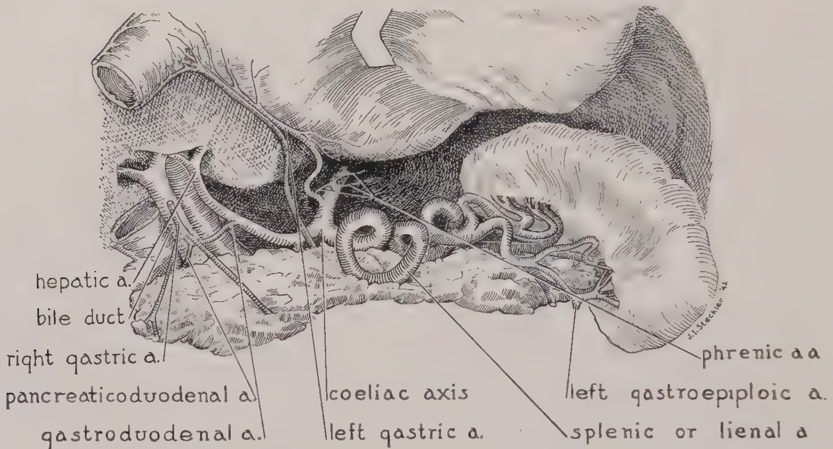
hepatic a.
bile duct
right gastric a.
pancreaticoduodenal a.
gastroduodenal a.
coeliac axis
left gastric a.
splenic or lienal a
left gastroepiploic a.
phrenic aa
J.I.Steckler '42

Figure 1

A more recent work by Michels ('42) on a composite study of the celiac axis, dealing in particular with the arterial bed and branching of the lienal artery, also presents a more limited discussion on tortuosity. Other less detailed references are found in Piquand ('10) in a study of terminal branchings cited by Michels, and Lipshutz in a paper on a composite study of the celiac axis ('17).

Michels and Springorum concluded that there is no correlation between arterial length and age of the subject, no correlation between diameter and length; both generalize, however, that the following factors may be influential: age depreciation, in individuals over 40 as a group, is evidenced by a loss of elasticity in the vascular system, therefore vessels may not return to their original length after stretching occurred as might conceivably be the case in a wandering spleen. It could be the result of a simple shortening of the luftline or no more than prolongation as a result of growth. Perhaps it is the result of sudden increase in blood pressure or of chronic hypertension.

OBSERVATIONS

(a) Gross description

Inasmuch as an index of tortuosity has already been presented in a previous work, the measurements and statistics presented here conform to that same method. The limited terminology used conforms to the common usage and is that applied in the Tulane laboratory.

The subject was a colored male, aged 84, whose clinical record listed arteriosclerosis, malnutrition, and dehydration as contributory factors in the death of the subject. The luftline measured 7 cm., the length of the artery 27 cm., giving an index of 386 which is 10% greater than any observed previously. The diameter of the artery at its origin was 7 mm.

Notwithstanding the convolutions of the artery, the lienal vein was normal, discounting any possibility of torsion of the pedicle attributable to a wandering spleen. The celiac axis in this subject arises from the front of the abdominal aorta in its natural position between the crura of the diaphragm just below the aortic hiatus, extending downward and forward about 2.25 cm. About 0.4 cm. from the origin of the celiac axis the inferior phrenic arteries arise. This is not the most common of the normal patterns for the inferior phrenic vessels and is noteworthy only in that respect. On the right side two branches arise in intimate association, while on the left there is definitely a single branch which subsequently divides. The left gastric artery is given off approximately 1.5 cm. from the origin of the celiac artery, and proceeds in a normal fashion. Other than the right gastroepiploic artery arising from the gastroduodenal, the hepatic artery is not noteworthy.

The course of the lienal artery becomes undulant immediately, coursing at first downward, forward, and to the left, crossing the lienal vein to the superior border of the pancreas, and at a distance of about 5 cm. curving upward and backward to make a close counterclockwise spiral, the descending arm of which passes anterior to its proximal ascending plane. This descending arm extends in a wide arc to the left, distinctly grooving the anterior surface of the body of the pancreas. It sweeps cranially to the superior border and describes a second smaller counterclockwise spiral which lies on the superior border of the pancreas. This second spiral is overlapped on the right by the first coil and in turn overlaps the third which lies behind and to the left. The second coil is the most closely bound and its descending arm in contradistinction to that of the first, passes posteriorly to the ascending. The artery now runs upward and to the left crossing the lienal vein, which at this point deeply grooves the superior border of the pancreas. The third and last coil is also counterclockwise and duplicates the first, the descending arm passing anterior to the ascending. However, it does not extend downward in a prolonged arc, but passes horizontally to the left along the superior border of the pancreas. Almost immediately after the third spiral, the lienal artery divides into superior and inferior terminal branches, the superior lying on a slightly superior plane to the other for the remainder of their course. These two branches run a short distance to the left (2 cm.) before turning sharply posteriorly and superiorly for a similarly short distance, when they reverse direction and run downward and forward again, crossing the lienal vein for the third time, at the tail of the pancreas. On this descending arm (3.5 cm.) the superior and inferior terminals each branch and turn superiorly. The penultimate branches undergo a subsequent division as they enter the hilus of the spleen.

The superior terminal occupies the upper half of an elongated hilus and is the source of the short gastric arteries. The inferior terminal occupies the lower half of the hilus and gives rise to the left gastroepiploic artery.

(b) Microscopic observation

The microscopic appearance of the artery in general is much what one would expect to find in an individual of 84 years, there being a relative increase in connective tissue at the expense of the elastic. Signs of arteriosclerosis and calcification are present although this pathology is not excessive. There is a general moderate thickening of the intima, although it is not constant in degree; and an irregular calcification around the internal elastic membrane in particular. No significance is attached to this as regards the tortuosity of the artery.

Pancreatic sections show deterioration in the isles of Langerhans which may be attributable to the fact that the material was not fresh and to the action of the embalming fluids. Furthermore, there is present a considerable increase in amount of connective tissue in excess of normal.

SUMMARY

A case of excessive lienal arterial tortuosity is described grossly and microscopically. Springorum's method of comparing tortuosity of such structures by an index is described. Using this method, this artery has the highest index of tortuosity yet observed, namely 386.

Although it has been both suggested and refuted by several observers that such tortuosity may be the result of a loss of elasticity with age, of long axis growth, of chronic hypertension, of arteriosclerosis, and of numerous other causes, no attempt is made here to discuss any of these theories, or on the other hand, to suggest one. Note is taken, however, of the advanced age of the subject (84), and that arteriosclerosis was a contributing factor in the death of this subject.

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THE NORMAL CHANGES IN THE POSITION OF THE EMBRYONIC KIDNEY

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TEN FIGURES

It has long been known that the kidney undergoes a remarkable change in position during early stages of its development. Most of the studies on this process were conducted in an effort to decide whether the kidney moves "actively," or is carried passively to its new position by other parts to which it is attached. However, the majority of the reports in the literature fail to indicate the mechanism of this active or passive movement held responsible by the respective authors. Preliminary studies suggested that the conditions involved in this process are too complex to be adequately described by one of these two terms without further explanation. The present detailed investigation was carried out in an effort to trace the mechanism of displacement of the kidney, as far as feasible with descriptive methods.

MATERIAL AND METHODS

From the author's collection of serially sectioned human embryos of 7.5 to 48 mm., the best suitable specimens were used to obtain graphic reconstructions of those organs which had to be considered as possibly influencing the position of the kidney. In these reconstructions, the adrenal, kidney, urogenital ridge, and umbilical artery of the right side appear projected on a sagittal plane, along with the liver and vertebral column. Reconstruction of the organs of one side was deemed sufficient since the changes in question are identical on both sides. Fourteen reconstructions were obtained from cross sections and one by superimposing sagittal sections. One of the 9-mm. embryos showed agenesis of the ureteric buds, as described previously ('39). The segments of the vertebral column were determined by counting from the first cervical segment, and also by the position of the first and last ribs. The original magnifications of the reconstructions varied from 15 to 50. In the early stages represented, the outlines of the adrenals are inaccurate because the boundaries of their early primordia are very indistinct. The segments occupied by the various organs were read from the reconstructions and compiled in curves (fig. 8). The vertical lines representing individual embryos in this figure are placed in such distances as to correspond very roughly to the time intervals during development.

In addition to this material, early stages of metanephros development were studied in embryos from the Minot Embryological Collection. A reconstruction was made of a 6.7-mm. embryo from that collection. However, it turned out that in these earliest stages the segments can not be determined with sufficient accuracy because of the low degree of differentiation of the vertebrae. For this reason, the results of the reconstruction of this 6.7-mm. embryo and the author's 7.5-mm. series are not entered in figure 8.

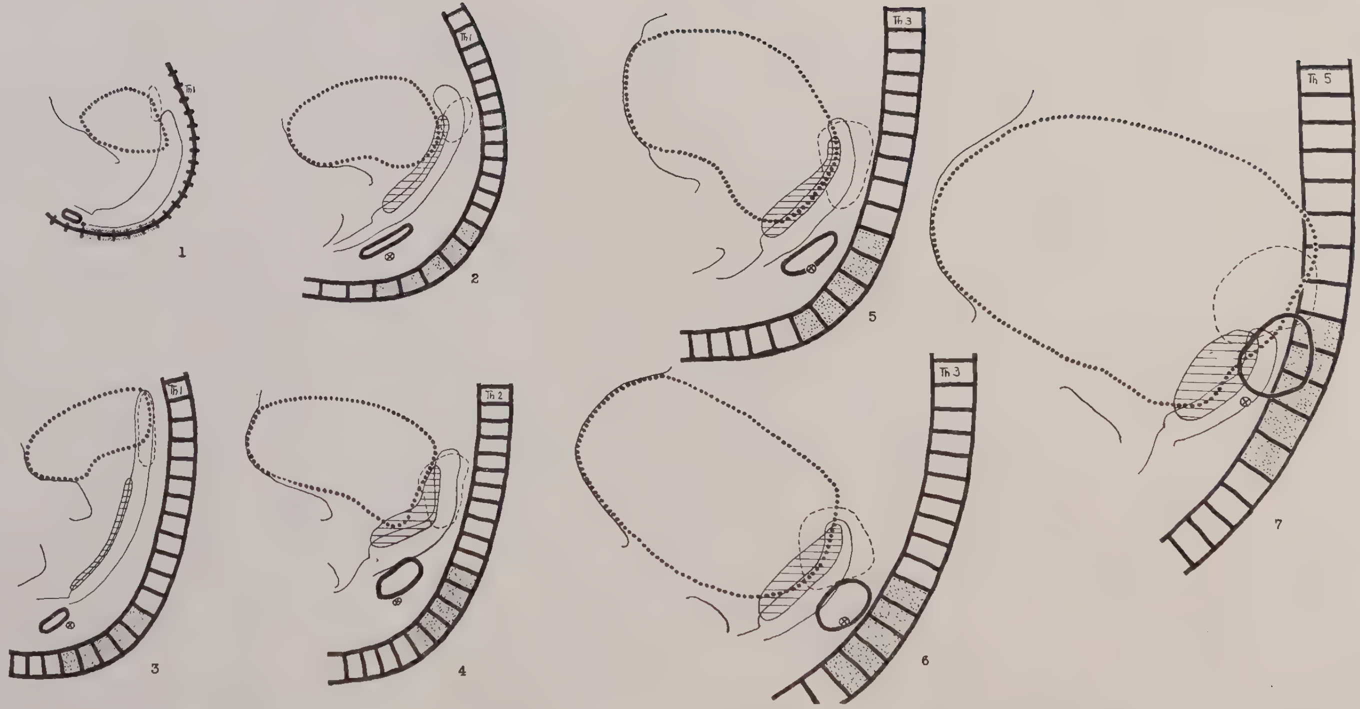
OBSERVATIONS

The following descriptions are based on the findings in the sixteen embryos whose organs were graphically reconstructed. The embryos will be described in groups representing the most important steps of the processes under consideration.

Embryos up to 7.5 mm. In embryos showing the earliest stages of metanephros formation, the caudal nephrons of the mesonephros are just differentiating and the adrenal cortex blastema appears with indefinite boundaries near the cranial portion of the mesonephros. The kidney is found caudal to the mesonephros. Its relation to the segments of the vertebral column can not be determined exactly by graphic reconstruction, because on cross sections the vertebrae of this region can not be definitely delimited from each other. However, it is certain that the kidney primordium arises at the level of the sacral segments, as described by Starkenstein. Approximate determination of the segments carried out in the reconstruction of a 6.7-mm. embryo, makes it probable that the kidney is located slightly farther caudally than in the embryos of the following group.

Embryos of 9 mm. (figs. 1 and 8). The axial organs of these embryos, as represented in figure 1 by the notochord, show a considerable and fairly uniform curvature throughout the trunk. The mesonephros is just reaching its full length and extends from the lower cervical to the lower lumbar segments. Near its cranial end appears the adrenal cortex primordium, not yet distinctly bounded. Its position is dorsal or dorso-cranial to the liver. The metanephros is found caudal to the mesonephros, and shows the dilated end of the ureteric bud beginning to form cranial and caudal extensions. It is located opposite the first and second sacral segments of the vertebral column, and ventro-caudal to the origin of the umbilical artery from the aorta.

Embryos of 9.5 and 10 mm. (figs. 2, 8, 10). In the lumbar and sacral regions the curvature of the vertebral column is approximately the same as in the previous stage, but the thoracic portion of the axial organs has straightened somewhat. The cranial border of the mesonephros shows the very beginning of a movement in the caudal direction which will be discussed later. The boundaries of the adrenal cortex are still indistinct. The curvature of the mesonephros is not, as in the previous stage, concentric with that of the vertebral column, but approaches the form of a chord. This is most probably an effect of the straightening of the axial organs, and will appear to a greater extent in the following stage (fig. 3). An instructive diagram of the changing curvature of the vertebral column may be found in the work of Bardeen ('05, fig. 44). If the axial organs and the mesonephros,



Figs. 1–7 Graphic reconstructions based on serial sections of human embryos of 9 mm. (fig. 1), 10 mm. (fig. 2), 10.5 mm. (fig. 3), 12 mm. (fig. 4), 15.5 mm. (fig. 5), 18 mm. (fig. 6) and 25 mm. (fig. 7). Vertebral column (in fig. 1: notochord): heavy lines, lumbar segments stippled; liver; dotted heavy line; ventral body wall and right mesonephros: thin lines; right gonad: horizontally lined; right adrenal: broken thin line; right kidney: heavy line; right umbilical artery at the aorta: ring with cross. The outlines of these organs appear projected on sagittal planes. × 9.

originally occupying concentric arches, straighten, the mesonephros will straighten more than the axial organs if it is to extend over the same number of segments. From this stage on, all embryos of the investigated period show a large amount of loose mesenchyme in the lumbo-sacral region, apparently filling the space between the diverging retroperitoneal and axial organs (figs. 2–7). In spite of this mechanism, the mesonephros and other viscera can not keep pace with the straightening vertebral column, and consequently the segmental extension of the viscera decreases during this and the following stage, as is apparent in figure 8. These changes have considerable bearing on the position of the small metanephros which is in close proximity with the caudal end of the mesonephros. It moves slightly in the cranial direction (fig. 8) and its longitudinal axis does not remain parallel to the vertebral column. This latter change is probably caused in part by the changed position of the mesonephros (fig. 2), and partly by the presence of the umbilical artery which interferes with the growth of the primordium in the cranial direction, and thus forces it to tilt. This relation of the metanephros to the umbilical artery is easily seen in reconstructions (fig. 2) and sagittal sections (fig. 10).

Embryos of 10.5 to 13.5 mm. (figs. 3, 4, 8). The changes observed in the embryos of this group bring the metanephros almost to its definitive position. Figure 8 shows that the viscera in the upper thoracic region, including liver, adrenal, and cranial border of the mesonephros, are uniformly displaced in the caudal direction. Detailed description and investigation of this change in the thoracic region are beyond the limits of the present work. (Here as in several other parts of the present report, the description is abbreviated by taking the vertebral column as a fixed scale, thus neglecting the influence of growth of the vertebral column itself.) In the lumbar and sacral regions, the structures under consideration show only slight changes in their segmental locations, with the exception of the kidney. Figure 8 shows the caudal border of the mesonephros and the umbilical artery moving slightly in the cranial direction, in continuation of the process described in the preceding paragraph. The caudal border of the kidney follows this movement, but the cranial border shows a steep cranial displacement between the stages of 10 and 11 mm. This is the expression of extensive growth of the kidney in the cranial direction alone. It can be explained on the basis of the above described tilting of the primordium. Its caudal end is turned toward the sacral vertebrae. It is fixed because the small amount of tissue between it and the skeleton can not yield. The cranial end, however, is free; hence all of the considerable longitudinal growth of the organ expresses itself as displacement of the cranial pole. Thus the center of the kidney passes the narrow space between the umbilical arteries. At this stage the kidney is a relatively thin oblong body (fig. 3). Immediately after its center has passed the umbilical artery, it begins to round itself out (fig. 4). It appears as if the kidney were mechanically forced to stretch temporarily in order to bypass the umbilical artery and leave the narrow pelvic region. This is, however, not a deformation of an existing mass of tissue, but rather an influence upon the direction of growth of a rapidly expanding primordium. At the end of this period, the cranial pole of the kidney has reached the lower border of the first lumbar segment and is thus almost in the position which it is to occupy during a long period of embryonic life (fig. 8).

Embryos of 15.5 to 21 mm. The most striking change during this period is the reduction of the segmental length of the mesonephros (fig. 8). There is only a slight variation in the position of the caudal end of this organ, but the cranial end moves from the eighth thoracic segment in the 15.5-mm. embryo to the second lumbar segment in the 21-mm. embryo. The role of degeneration of the cranial portion of the mesonephros in these changes will not be discussed here. There is also a displacement of liver and adrenal cortex during the same period, but to a far lesser degree. The kidney rounds itself out as described in the previous section. The result is, that its cranial pole keeps approximately the same distance from the umbilical artery during the previous and present stages (13.5 to 21 mm.), whereas the caudal pole undergoes a relative movement in the cranial direction and thus approaches the level of the umbilical artery (fig. 8). In the 18-mm. embryo the kidney is found in contact with the adrenal for the first time. Figure 8 shows that this contact is not established by cranial movements of the kidney, but by caudal displacement of the adrenal (see above). In the 21-mm. embryo the adrenal occupies the most caudal position encountered in the present series of stages.

Embryos of 25 to 48 mm. The only organ under consideration which still undergoes considerable displacement during this late period is the mesonephros. It is now attached to the inguinal region by a well developed gubernaculum and there can be little doubt that this attachment accounts for the removal of the mesonephros from the posterior abdominal wall. A detailed description of development and action of the gubernaculum in the human embryo was given by Moszkowicz ('35). The cranial borders of liver and adrenal keep their position at the lower borders of the seventh and tenth thoracic segments, respectively, while the caudal border of the adrenal and the cranial pole of the kidney show a slight cranial displacement. It appears as if these changes were due to a relative decrease in size of the adrenal. The kidney is now definitely rounded; this rounding and the slight cranial displacement just mentioned have resulted in bringing the lower pole of the organ to a level cranial to that of the umbilical artery. It appears that a slight caudal displacement of the artery, whose mechanism was not determined, also plays a role in this process (fig. 8). During the oldest period investigated (25 to 48 mm.) most organs under consideration have reached a stabile position. It can only be conjectured at the present time that the slight further ascensus which the kidney has to undergo in order to reach its final position, may be caused by a relative decrease in size of liver and adrenal, with the cranial border of the liver as the fixed point and the kidney remaining in contact with the lower surface of the adrenal.

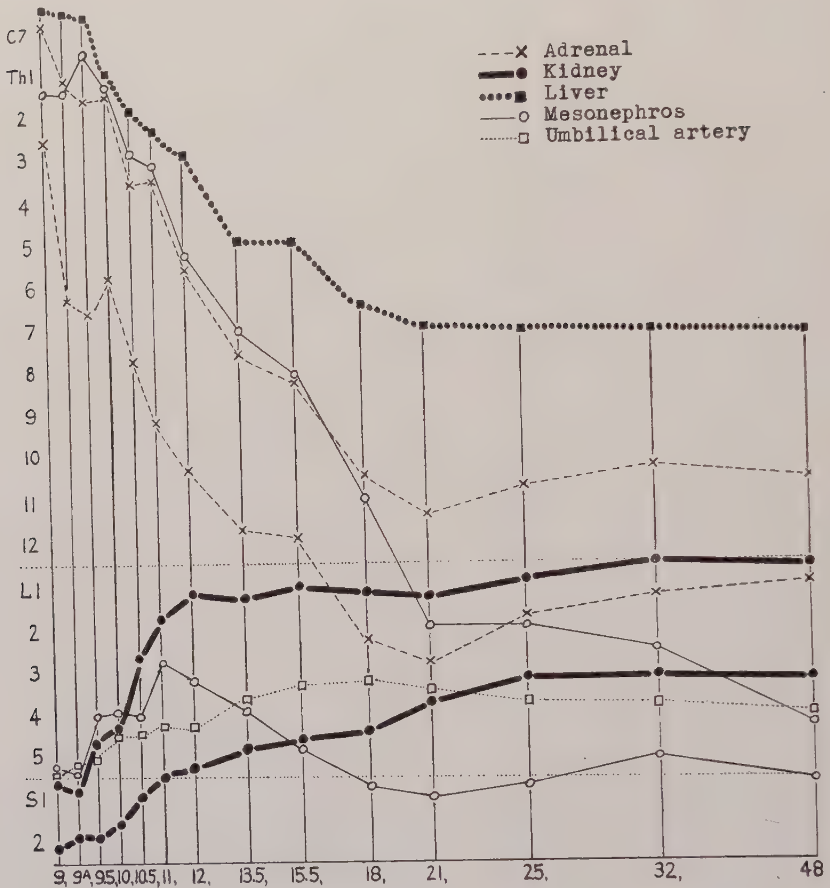

---x Adrenal
—● Kidney
••••■ Liver
—o Mesonephros
·······□ Umbilical artery
C7
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fig. 8

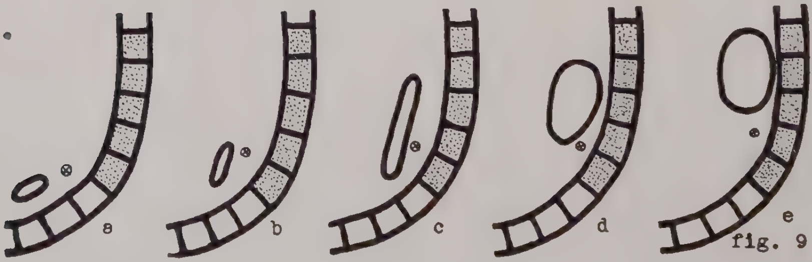
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fig. 9

DISCUSSION

In his classic description of the development of the urogenital tract Felix ('12) holds that the kidney primordium moves craniad by growth of the ureteric bud until the end of that bud begins to branch. By this time, that is, in embryos of 9.5–13 mm., the pelvis of the kidney has reached its definitive position opposite the second lumbar vertebra. Further growth of the ureter occurs only as far as is necessary to keep up with the increasing distance of its end points. In other words, Felix assumes that the kidney reaches its final position after a brief period of active migration by growth of the ureteric bud. This opinion is reflected in most of the text-books.

Brockmann ('36) traced the position of the kidney in human embryos and arrived at a different conclusion. He holds that straightening of the body causes a change in relations, resulting in a displacement of the kidney in the cranial direction when compared with the vertebral column. A similar mechanism was hinted above in the description of embryos of 9.5 and 10 mm. Starkenstein ('38) opposes Brockmann's conclusion and points out that Brockmann failed to examine the earliest stages during which most of the displacement occurs. He advocates active ascensus of the kidney without specifying how the organ accomplishes active movement. Brockmann maintains in a second publication ('38) his previous view for the stages which he had investigated, and objects to Starkenstein's failure to differentiate between migration and displacement.

Fig. 8 Graphic presentation of the position of the right kidney, adrenal and mesonephros (cranial and caudal borders), liver (cranial border) and the level of origin of the right umbilical artery from the aorta, after graphic sagittal reconstructions. Each vertical line connects all points related to one embryo whose length in millimeters is given at the bottom. These lines are placed in distances very roughly proportionate to the time intervals during development. The embryo designated by 9 A shows agenesis of the ureteric buds (Gruenwald, '39). In this case the kidney measurements refer to the metanephric blastema alone.

Fig. 9 Diagrams illustrating the displacement of the kidney in relation to vertebral column and umbilical artery. Changes in the curvature of the vertebral column are not considered. The diagrams represent approximately the following stages: a, 7 mm.; b, 10 mm.; c, 11 mm.; d, 18 mm.; e, 25 mm. Drawn as figures 1–7.

Gruenwald ('38) points out that the ureteric bud is covered with a thick cap of nephrogenic tissue from its earliest stages. This makes a displacement of the whole primordium by growth of the ureter alone (Felix) highly improbable. A later finding ('39) showed that in a 9-mm. human embryo with agenesis of the ureteric buds the nephrogenic tissue had moved away from the caudal end of the wolffian duct exactly like a normal kidney primordium (see also the present fig. 8, embryo 9 A). This disproves an influence of the ureter upon the position of the kidney in those early stages in which the primordium might be small enough to yield to pressure by the ureter.

As suggested in the introduction to the present report, no uniform explanation should necessarily be expected for all phases of displacement of the kidney primordium. It was found that up to the 10-mm. stage the kidney keeps a constant relation to the caudal pole of the mesonephros while moving craniad. This is strongly in favor of displacement by differential growth as against "active" movement. As suggested in the description of the observations, straightening of the body is probably the cause of this change, in a similar manner as suggested by Brockmann for later stages. As soon as the kidney reaches the umbilical (common iliac) artery, it tilts and thus loses its previous close relation to the mesonephros. Its caudal end is now directed dorso-caudad toward the sacral vertebrae, and thus prevented from moving caudad again when the urogenital ridge begins its descensus at the 13.5-mm. stage (fig. 8). For the same reason the caudal pole does not yield during the following period of rapid growth of the kidney, and the cranial pole must move upward that much more (fig. 8). In this manner the pelvis of the kidney reaches approximately its final position, and rounding and relative shortening finally remove the kidney completely from the crotch of the umbilical arteries. The remaining slight cranial displacement is probably the result of a relative decrease in size of the liver and adrenal. These latest stages are not covered by the present investigation.

According to the present view all displacement of the kidney is effected by differential growth, although not in the simple and uniform manner as suggested by previous investigators. Participation of active ascensus (Starkenstein) appeared improbable from the beginning since no mechanism is known by which an organ of the structure of the kidney can move actively. The only process which might be considered as active movement of the primordium, is displacement by pressure of the growing ureter. It is obvious that this could only occur in very early stages, but the above quoted findings in agenesis of the ureteric buds exclude this possibility, as does the constant relation of kidney and mesonephros in the stages in question.

In conclusion, the ascensus of the kidney can be described as follows (fig. 9). In embryos up to 10 mm. the retroperitoneal tissues of the lumbar and sacral regions, including mesonephros and kidney, move in the cranial direction (fig. 9 a, b). Shortly before this movement comes to an end, the upper pole of the kidney deviates ventrally (fig. 9 b). In this new position the caudal pole of the kidney is directed against the anterior surface of the sacral vertebrae and thus prevented from moving downward. There follows a period of very fast lengthening of the kidney (10–11 mm., fig. 9 c) which, according to this peculiar position of the organ, can result only in cranial displacement, with the caudal pole remaining in a constant position. In the course of this growth period the pelvis of the kidney passes the level of the umbilical arteries. The shape of the kidney changes now, perhaps because the organ has emerged from the narrow passage between these arteries. The transverse diameter increases simultaneously with a decrease in length (fig. 9 d). The kidney thus rounds itself out and the caudal pole moves craniad until it reaches the level of the umbilical arteries (21-mm. embryo). Meanwhile the kidney has come in contact with the adrenal (18-mm. embryo) and moves now, along with the caudal border of the adrenal, slightly craniad (fig. 9 e). The nature of this last slight movement was not ascertained; changes in the size of liver and adrenal may play a role. In the 25-mm. embryo the kidney is found entirely above the level of the umbilical arteries, and keeps a constant position in the upper three lumbar segments during the remainder of the period covered by the present investigation.

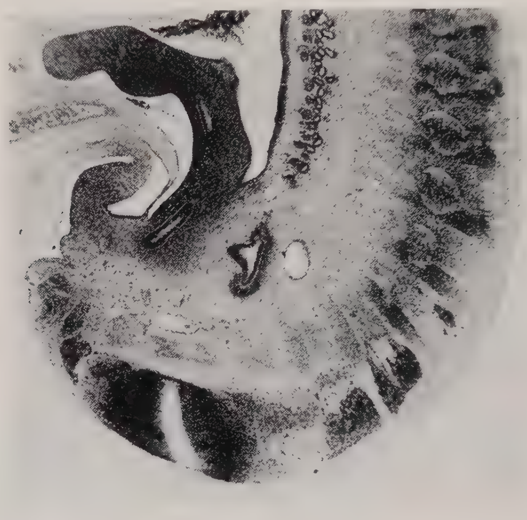

Fig. 10 Parasagittal section of a 10-mm. human embryo (see also fig. 2). Azan stain.

FAILURE OF NORMAL DISPLACEMENT: PELVIC KIDNEY

Most authors regard congenital pelvic kidney as the result of arrested development, that is, retention of the kidney in its early embryonic position. Laughlin ('42) lately disputed this concept and held that in the malformation in question the kidney is pulled down into the pelvis from the lumbar region by abnormal attachment to organs moving in that direction. He based his theory on Hinman's ('35) statement that the kidney is found in embryos of 9–10 mm. in its final position with its pelvis at the level of the second lumbar vertebra, and that from that time on other structures move away from the kidney in the caudal direction. It is obvious that Hinman failed to consider sufficiently early stages. The present findings show, in accordance with the data given by Starkenstein, that the kidney primordium is at first located in the pelvis, at the level of the sacral segments. In the course of its cranial displacement it must pass the crotch of the umbilical arteries. This is important from the viewpoint of teratology not only, as Lewis and Papez point out, because the two kidney primordia come very close to one another and may fuse, but also because the kidneys can pass the arteries only after tilting as described here. Failure of this complicated movement would trap them below the umbilical arteries and keep them in the pelvis. The resulting condition would be pelvic kidney. We can thus describe the formal genesis of that malformation as an arrest of displacement of the kidney, favored by the peculiar relations to the umbilical (later common iliac) arteries or failure of the early kidney primordium to follow the caudal end of the mesonephros in its cranial movement.

SUMMARY

The relation of the kidney primordia to the surrounding organs was examined in human embryos of 6 to 48 mm., mostly by means of graphic reconstruction from transverse serial sections.

The kidney moves craniad from its original position in the sacral region, along with the caudal portion of the mesonephros. This displacement is probably due to a diminution of the body curvature. The cranial pole of the kidney reaches the umbilical artery and avoids it by deviating ventrally. The caudal pole is thus directed dorso-caudad toward the sacral vertebrae (10-mm. embryo).

The following rapid growth of the kidney causes its pelvis to pass the umbilical artery in the cranial direction because the caudal pole cannot yield (11-mm. embryo). The kidney then rounds itself out and shortens relatively, thus escaping the crotch of the umbilical arteries in the cranial direction (21-mm. embryo). A slight further movement in the same direction brings it into the upper three lumbar segments (25-mm. embryo) where it remains for the rest of the period investigated.

No active cranial migration of the kidney takes place at any time.

Congenital pelvic kidney is the result of failure of the organ to undergo its normal change of position. The obstacle of the umbilical artery favors this arrest of displacement.

I wish to acknowledge the generosity of the Department of Anatomy, Harvard Medical School, in permitting me to use the Minot Embryological Collection.

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THE EFFECTS OF ANTIGONADOTROPIC SERUM ON THE EARLY POSTNATAL DEVELOPMENT OF THE REPRODUCTIVE SYSTEM OF THE MALE RAT[1]

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TWO TEXT FIGURES AND TWO PLATES (TWELVE FIGURES)

The atrophy of the gonads and the accessory organs following hypophysectomy has been repeatedly observed for mammals and lower vertebrates. The dependence of the gonads of infantile mammals upon the pituitary gonadotropic substances has not been demonstrated. This has resulted, in part at least, from the difficulties of surgical procedures in very young animals.

With the advent of the antihormone concept, reviewed by Collip et al. ('35) and Thompson ('41), a method was suggested for hypophysectomizing an animal immunologically without surgical procedures. An animal could be injected over long periods of time with a serum containing aspecific antihormones, thus passively neutralizing the animal's own pituitary secretions. Sera containing aspecific gonadotropic antihormones have been produced in animals by several investigators as a result of repeated injections of gonadotropic hormones. Rowlands ('37) and Fluhman ('36) showed that injections of these sera lead to atrophy of the reproductive systems of older animals similar to that following surgical

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hypophysectomy. Thus it was thought that similar treatment of infantile male rats might reveal some relationships between the gonads and pituitary gland hitherto undemonstrated for mammals of this age.

MATERIALS AND METHODS

Litters of healthy newborn rats were selected on the day of birth and the males divided into two groups. One group from each litter served as experimentals and the other group as littermate controls. The experimental rats were marked by tying the tails near the base with thread and then amputating them just distal to the thread.

The serum used was obtained from the blood of a pony which had been injected with aqueous extracts of sheep pituitary glands for 30 to 38 months. In some cases, the blood was allowed to clot normally and the serum expressed from the clot was separated by centrifugation. In other cases, the blood was drawn into sodium citrate solution to prevent clotting. The corpuscles were separated either by centrifugation or by allowing them to settle while in the refrigerator. The plasma thus obtained was made to clot by the addition of an amount of calcium chloride solution equivalent to the sodium citrate originally used. The coagulated fibrin was removed and the serum was dialyzed against distilled water to remove the salts which had been added. The euglobulin protein precipitating during the dialysis was removed, and the remaining solution was dialyzed against physiological saline to render it again isotonic. Regardless of the method of obtaining the serum, it was Seitz-filtered and aseptically stored in serum bottles. These bottles of serum were kept frozen in the refrigerator except for the short time necessary to remove some serum for use.

The antigonadotropic effect of the serum was such that 2.0 ml. inhibited an amount of sheep pituitary extract which produced 80 mg. ovaries when assayed in 21-day-old female rats. The serum was aspecific since it inhibited the gonadotropins of the pituitary glands of other species, including the rat (unpublished data). When tested for antithyrotropic activity, the serum failed to produce any inhibition of the increased oxygen consumption of adult rats resulting from the injections of the extract of sheep pituitary glands.

The serum was injected subcutaneously each day for 10 or 20 days. The volume of serum each day was gradually increased with the growth of the rats so that a total volume of 2.2 ml. and 6.1 ml. was given during the 10- and 20-day periods respectively. The rats were autopsied on the day following the last injection. The organs removed were first weighed on an analytical balance and then fixed in Bouin's fluid. As a variation from the above procedures, one group of rats was injected from the day of birth to the tenth day and was then allowed to go for 10 days without injection before being autopsied on the twenty-first day of life.

Control data consisted of two types: data from uninjected littermate rats autopsied at the same time as the experimental rats, and data from rats injected with normal horse serum having no antigonadotropic effect. Since the tails of the injected rats were amputated as a means of marking them, the effects of tail amputation alone were determined.

RESULTS

The data summarized in table 1 are largely self-explanatory. The weights of certain organs of day-old rats were not determined because of the difficulty of uniformly complete removal and the doubtful significance of such small weights.

If one compares the organ weights of rats with amputated tails and the organ weights of untreated littermates, no significant differences are seen. Thus it can be concluded that amputation of the tails for the purpose of marking the rats had no effect upon the organ weights. Since no result of amputation was observed in 10 days, it seems highly improbable that one would appear in a longer time.

Injections of normal horse serum resulted in an apparent inhibition of body, testis and thymus growth. When the testis

TABLE 1

The effects of various treatments on the body and endocrine gland weights of immature male rats.

AGE IN DAYS AT AUTOPSY	TREATMENT	WEIGHT OF							
		Body[1]	Testes	Seminal vesicles	Prostate	Thyroid	Thymus	Adrenal	Pituitary gland
1	None	5.4 (10)	5.1 (10)	1.0 (10)	1.0 (10)	....	....	....	....
11	None	19 (6)	33 (6)	3.4 (6)	4.9 (6)	2.8 (6)	59 (6)	4.1 (6)	1.5 (6)
	Tail amputation	18 (5)	36 (5)	3.2 (5)	4.6 (5)	2.7 (5)	53 (5)	4.1 (5)	1.5 (5)
11	None	23 (9)	50 (9)	3.3 (9)	5.8 (9)	3.1 (9)	73 (9)	5.3 (9)	1.3 (9)
	Normal serum	21 (14)	39 (14)	3.2 (14)	5.9 (14)	2.8 (14)	55 (14)	4.9 (14)	1.2 (14)
11	None	24 (15)	45 (15)	3.4 (15)	5.3 (15)	3.1 (15)	71 (15)	6.2 (15)	1.6 (15)
	Antiserum	20 (21)	25 (21)	1.6 (21)	3.3 (21)	2.2 (21)	50 (21)	5.5 (21)	1.5 (21)
21	None	48 (4)	208 (4)	7.6 (4)	32 (4)	6.5 (4)	217 (4)	16 (4)	2.5 (4)
	Antiserum	38 (6)	88 (6)	4.5 (6)	17 (6)	5.3 (6)	140 (6)	14 (6)	3.1 (6)
21[2]	None	44 (54)	192 (54)	6.9 (46)	31 (42)	5.2 (22)	172 (22)	14.3 (22)	2.2 (42)
	Antiserum	37 (30)	62 (30)	3.8 (30)	20 (30)	....	....	....	2.4 (30)
21	None	46 (13)	195 (13)	7.6 (13)	30 (13)	5.8 (13)	190 (13)	15.9 (13)	2.4 (13)
	Antiserum 1st 10 days	41 (19)	171 (19)	14.7 (19)	40 (19)	5.3 (19)	149 (19)	13.0 (19)	2.2 (19)

[1] The body weights are given in grams; all other weights are in milligrams. The numbers in parentheses indicate the numbers of rats from which the averages were obtained.

[2] The experimental and control rats of this group were not littermates.

and thymus weights were calculated as percentage of body weight, these differences disappeared. Whether this inhibition of body growth is merely incidental to disturbance of the young or is a general response to the injected serum proteins has not been determined.

When an actively antigonadotropic serum is injected for 10 days, a real inhibition of testis, seminal vesicle and prostate weights is produced. It should be pointed out at this time that the depressions in thyroid and adrenal weights of all groups are of doubtful significance, and no attempt will be made to interpret the changes in thymus weights.

If the injections are continued for 20 days, the depression of the testis, seminal vesicle and prostate weights of treated animals is in the same direction but is of greater magnitude. The pituitary glands of the treated rats, however, are increased in weight.

In order to test whether or not the hypertrophied pituitary gland was in a hypersecretory state, rats were injected for 10 days and autopsied at 21 days of age. The data in table 1 show that the seminal vesicles and prostates were larger than those of controls, but the testes and pituitary glands were slightly smaller than normal. These results appear contradictory since the seminal vesicle data support the hypersecretory concept and the data on testis growth do not. An interpretation is offered later whereby these two groups of data can be brought into agreement.

Although the amount of partial descent of the testes in 11-day-old rats and the age of complete descent of the testes in the 21-day-old rats were determined, the data are not given in tabular form. It suffices to say that the variations between individuals of the same litter made the small differences obtained quite insignificant.

If the data are graphically represented, some interesting facts appear. In figure 1 are given the curves which result when the data are plotted as graphs. It can be seen that the body growth of untreated rats is rather uniform for at least 21 days. When rats are injected with antigonadotropic serum,

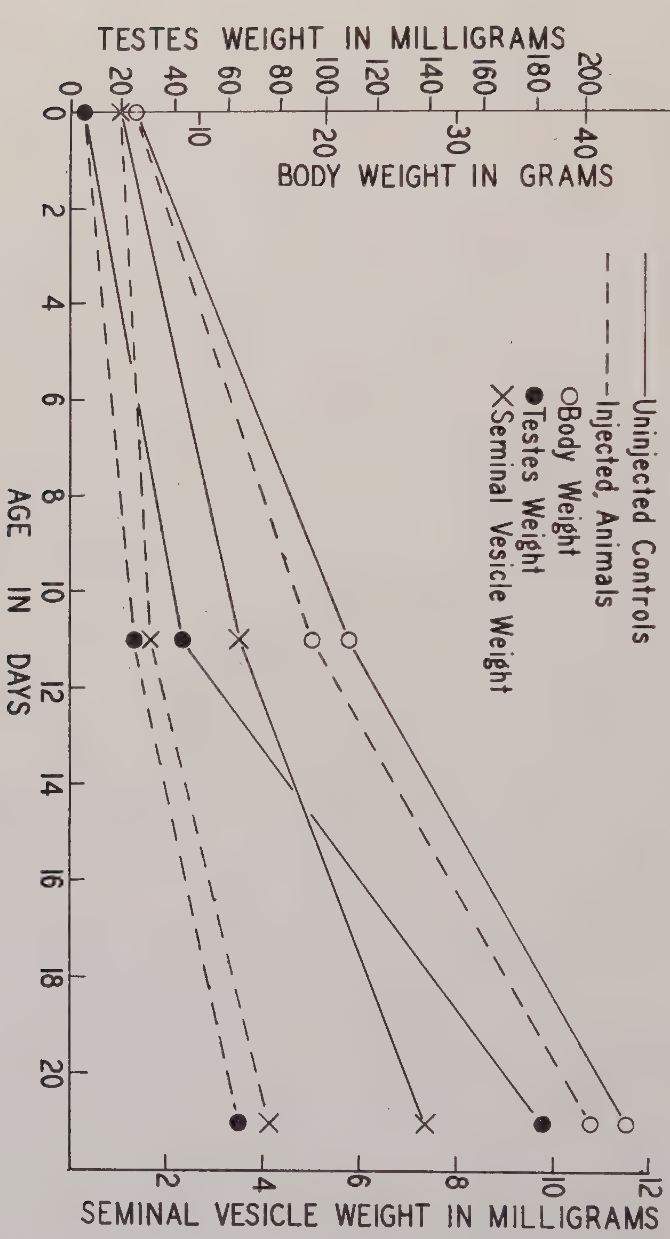

Fig. 1 Effects of antigonadotropic serum on the body and endocrine gland weights of 11-day-old and 21-day-old male rats.

the growth is uniform but slightly depressed. The rate of normal testis growth is increased after the eleventh day of age. This may be the result of an increased sensitivity of the testis to the gonadotropic hormone, or it may correspond to the onset of secretion of this hormone by the pituitary gland. The increase in growth rate does not occur if the animals are injected with antigonadotropic serum. The seminal vesicles increase in size at a fairly uniform rate, and this rate of growth is depressed by the serum injections. This decrease in rate of growth is more than would result from the slight inhibition of body growth since corrections of the seminal vesicle weights to percentage of body weight show a relative, as well as an absolute, depression of growth.

The data obtained from rats injected for only the first 10 days of life and killed on the twenty-first day are graphically shown in figure 2. The testes of the experimental rats apparently grow during the 10 days without treatment at a rate equal to that of control rats. This may mean that the depressed androgen production during the 10 days of injection, as indicated by smaller seminal vesicles, has not resulted in a castration-like hypersecretion of the pituitary gonadotropic hormone. An alternative explanation is that the testes of the rats are growing at a maximum rate between the tenth and twentieth days and cannot respond by increased growth to an increased gonadotropin (FSH), even if present. The latter hypothesis is supported by the curve of seminal vesicle growth which indicates that an excessive amount of gonadotropin (LH) is indirectly stimulating the accessory glands during the last 10 days of the experiment. It seems unlikely that a castration-like hypersecretion of one factor of the gonadotropic complex would occur without an increase in the secretion of the other.

HISTOLOGICAL RESULTS

The tissues to be studied histologically were fixed in Bouin's fluid at the time of autopsy, embedded in paraffin, cut at 6 μ to 10 μ, and stained with hematoxylin and eosin. In all cases only tissues taken from littermate rats were compared.

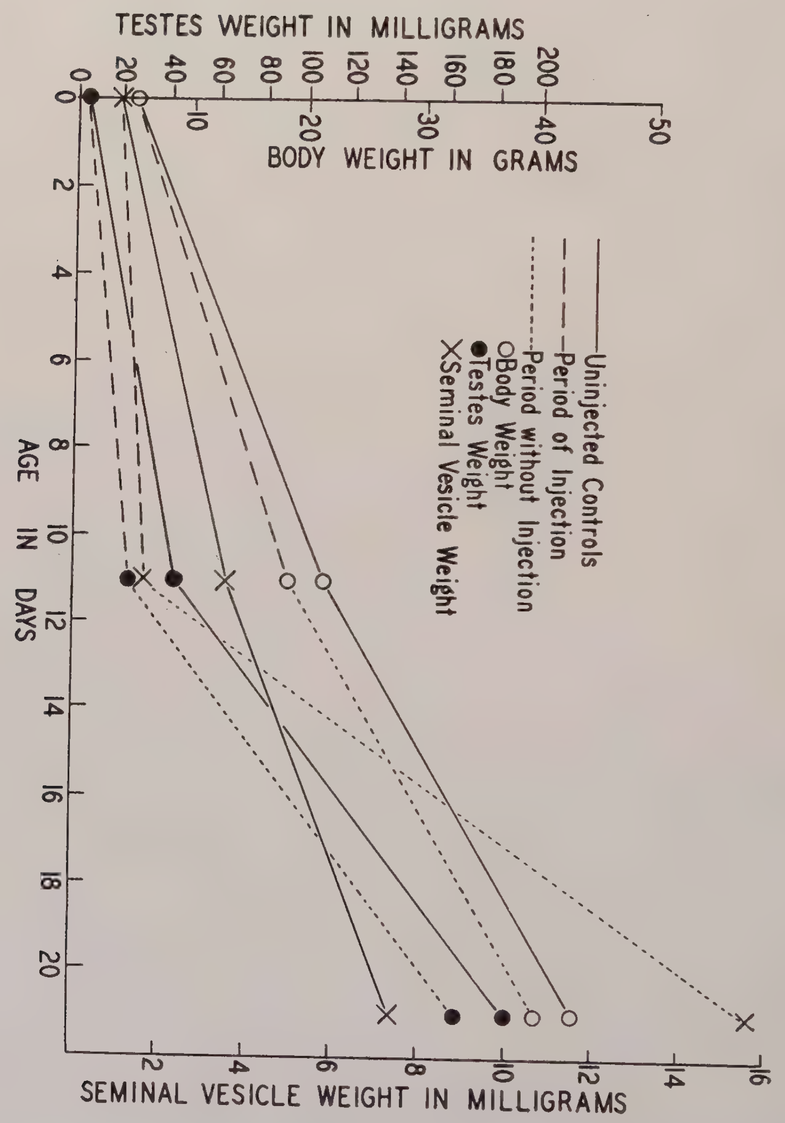

Fig. 2 The body and endocrine gland weights of 21-day-old male rats injected from the first to the tenth day of life with antigonadotropic serum.

In figure 3 are shown the tubules of the testis of an untreated 11-day-old rat. At this stage the lumen has not formed and there is a definite tendency toward a doubling of the number of cell layers in the tubular wall. When compared with figure 4, no very striking differences are seen. The diameters of the tubules are much the same. Those of the treated animals are more densely packed and this fact alone may account for part of the difference in the weights of the two organs. Comparison of figures 5 and 6 shows that treatment with antiserum results in an inhibition of histological development as well as the gross weight of the seminal vesicles. Those of treated animals lack the secondary and tertiary branches of the secretory tubules. Two photomicrographs cannot show the full extent of the difference but serial observation of the sections makes the differences quite apparent.

In figure 7 are shown the seminiferous tubules of a normal 21-day-old rat. The differentiation of the cell types in the tubules has progressed with the increase in diameter. In figure 8 are the tubules of a littermate treated daily with antigonadotropic serum. The increase in diameter, formation of a lumen and the cellular differentiation have been inhibited. Here again the tubules appear to be much more compactly arranged than in the testis of the normal littermate. The differences between the seminal vesicles shown in figures 9 and 10 are quite apparent. Those of the treated male, although somewhat larger in size, show little advancement beyond the 11-day stage.

In figures 11 and 12 are shown the seminiferous tubules of a 21-day-old control and those of a littermate rat which had been injected for the first 10 days of life with antigonadotropic serum. Although they are not identical, the similarity is very striking. The cellular differentiation of the tubule in the injected animal is practically at the same stage as in the uninjected littermate. The difference in compactness of the tubules is much less marked than in the preceding cases. The seminal vesicle (fig. 14) of the treated rat is greatly advanced over that of the control (fig. 13). Secretion can be seen in the lumina of the secretory tubules, although Moore et al. ('30) state that such secretion normally does not appear until the forty-fifth day of age.

Differential counts of the cells of the pituitary glands were made by Dr. John C. Finerty, and the relative percentages of acidophilic, basophilic, and chromophobic cells were determined for three rats from each type of experiment. There was an increase in basophilic cells from an average of 5% for normals to 21.7% in treated rats on the day following the tenth daily injection. Untreated 21-day-old rats had an average of 9.4% basophiles but 20 days of treatment increased this to 18.7%. When the rats were injected for only the first 10 days, 7.8% of the pituitary cells were basophiles at 21 days of age. In addition to the basophilic changes, antiserum resulted in a definite reversible decrease in the acidophile cells which cannot be interpreted at the present time.

DISCUSSION

The various types of data given with the results can be brought into agreement as a working hypothesis. For the sake of convenience, the follicle-stimulating (FSH) and the luteinizing (LH) factors of the pituitary gonadotropic complex will be considered separately. The selective action of these factors on seminiferous tubules and interstitial cells has been well established by Evans et al. ('34), Smith et al. ('34) and Greep et al. ('36).

The LH from the pituitary gland stimulates the interstitial cells of the infantile testis to secrete androgen. Since Womack and Koch ('32) have obtained androgen from the fetal testis, this stimulation of androgen production may extend well into prenatal life. When antigonadotropic serum is injected into male rats, the LH is prevented from stimulating the interstitial cells and seminal vesicle growth is retarded. This retardation is not complete and the finding is in agreement with that of Price ('36). The small amount of growth which does occur may depend upon one or more factors. Howard

('41) believes that this growth of accessories which occurs in the absence of testicular influence is a result of stimulation by androgens secreted by the adrenals.

An inspection of the testes growth curves indicates that an increase in the rate of growth occurs about the eleventh day of life. This suggests that the FSH may be first secreted at this time from the pituitary gland, or that there is a sudden increase in the amount being secreted. Since Clark ('35) finds gonadotropic activity present in the pituitary glands of male rats at the day of birth, and that the pituitary potency increases gradually until the rats are 50 days of age, these two explanations appear to be untenable. Another explanation is that the seminiferous tubules develop independently of the pituitary FSH stimulation for about the first 10 days. After that they become reactive to the FSH and begin to differentiate at a maximum rate. That spermatogenesis is independent for a time is supported by our evidence from the experiments with antihormone. The antihormone injected during the first 10 days of life inhibited the testis weight and interstitial cell activity, but failed to prevent the differentiation of the cellular elements of the tubule. If the injections were continued through the twentieth day of life, the tubules develop very little beyond the 11-day stage. Although Moore ('36) presented evidence showing that spermatogenesis could not be accelerated and suggested that spermatogenesis in the rat goes on at a maximum rate, our evidence in favor of this is yet only circumstantial.

It is also suggested that the androgen being secreted by the infantile testis is acting as a brake on the pituitary gland even during the early postnatal life. When the androgen level is decreased by antihormone injections, the brake is reduced and the pituitary gland becomes hyperactive and increasingly basophilic. If the antihormone injections are stopped after the tenth day, the androgen secreted from the interstitial cells as a result of LH activity, stimulates the seminal vesicles to hypertrophy and also results in a reduction of the basophilism of the pituitary gland. It is tentatively suggested that the excessive amounts of FSH which may be present are not obviously manifested because the maximally growing testis cannot respond by excessive growth.

In conclusion it may be said that the results obtained and the conclusions derived bring into reasonable agreement the several lines of evidence relating to this problem. Certain aspects must be substantiated by additional and alternative investigations. It is thought that gonadotropic antihormone will be very useful in the solution of these problems.

SUMMARY

1. Injections of antigonadotropic serum into male rats from the first to the eleventh day of life result in a small inhibition of testis growth, and definitely retard the growth of the accessory reproductive organs.

2. Continuation of these injections through the twentieth day of life prevents the increase in testicular growth rate which normally occurs, and continues to inhibit the growth of the male accessory glands.

3. Histologically the differentiation of the seminiferous tubules of injected rats is not materially inhibited during the first 11 days of injection, and this suggests that the gonadotropic hormone is not necessary for this amount of development.

4. Differentiation beyond the 11-day stage, however, is almost completely inhibited by the antihormone.

5. Antihormone treatment for the first 10 or the first 20 days of life results in about the same degree of pituitary basophilism.

6. The gonadotropic secretion of the pituitary gland in the male rat is under the control of gonadal hormones as early as the tenth day of life.

7. It is suggested that the rate of tubular differentiation and growth in the testis is maximal in the rat during the early postnatal period.

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PLATE 1

EXPLANATION OF FIGURES

3 Seminiferous tubules of an untreated 11-day-old rat (rat no. 2, litter no. 52). × 267.

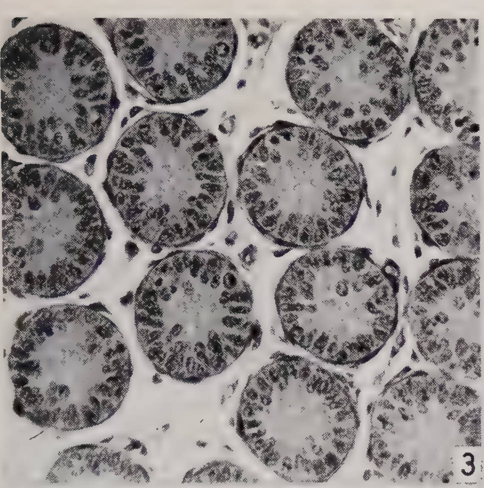
4 Seminiferous tubules of an 11-day-old rat (rat no. 4, litter no. 52) which had received ten injections of antigonadotropic serum. × 267.

5 Seminal vesicle of an untreated 11-day-old rat (rat no. 2, litter no. 52). × 45.

6 Seminal vesicle of an 11-day-old rat (rat no. 4, litter no. 52) which had received ten injections of antigonadotropic serum. × 45.

7 Seminiferous tubules of an untreated 21-day-old rat (rat no. 1, litter no. 35). × 267.

8 Seminiferous tubules of a 21-day-old rat (rat no. 4, litter no. 35) which had received twenty injections of antigonadotropic serum. × 267.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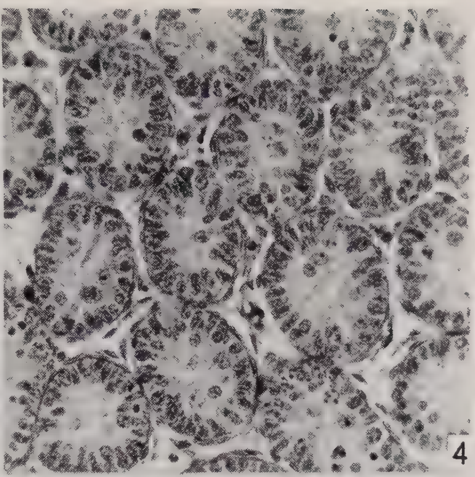
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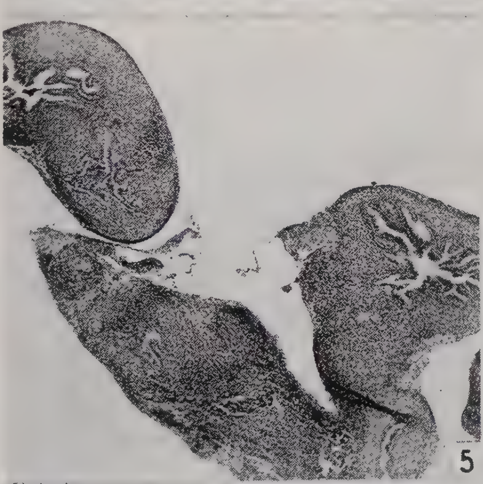
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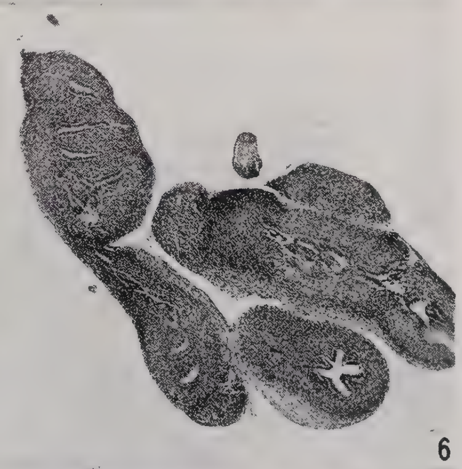
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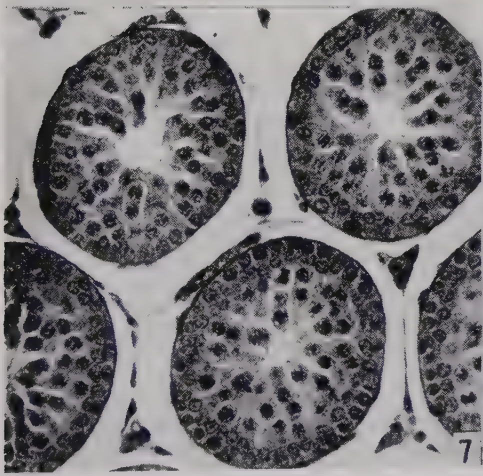
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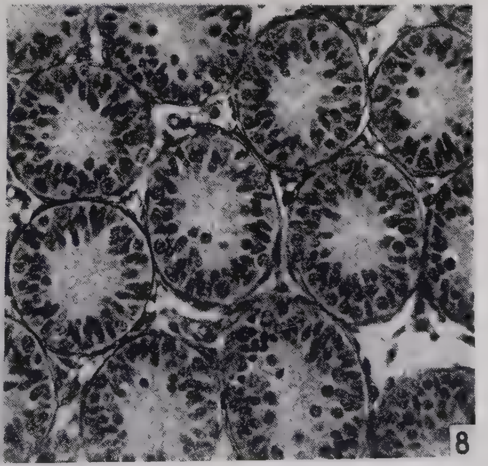
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PLATE 2

EXPLANATION OF FIGURES

9 Seminal vesicle of an untreated 21-day-old rat (rat no. 1, litter no. 35). × 45.

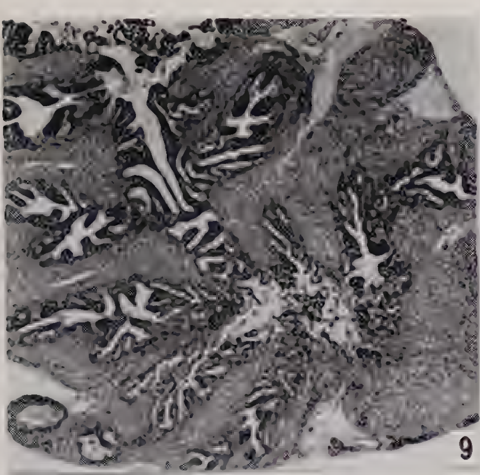
10 Seminal vesicle of a 21-day-old rat (rat no. 4, litter no. 35) which had received twenty injections of antigonadotropic serum. × 45.

11 Seminiferous tubules of an untreated 21-day-old rat (rat no. 1, litter no. 42). × 267.

12 Seminiferous tubules of a 21-day-old rat (rat no. 2, litter no. 42) which had received only ten injections of antigonadotropic serum during only the first 10 days of life. × 267.

13 Seminal vesicle of an untreated 21-day-old rat (rat no. 1, litter no. 42). × 45.

14 Seminal vesicle of a 21-day-old rat (rat no. 2, litter no. 42) which had received only ten injections of antigonadotropic serum during only the first 10 days of life. × 45.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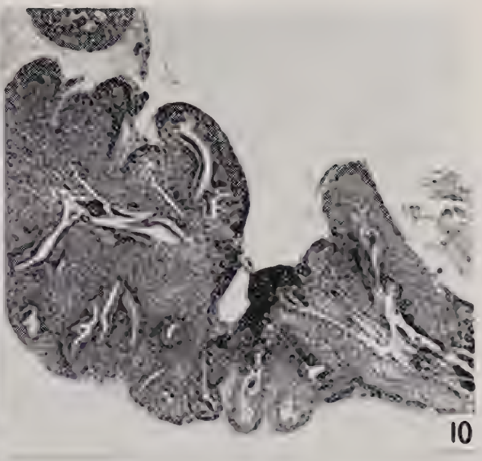
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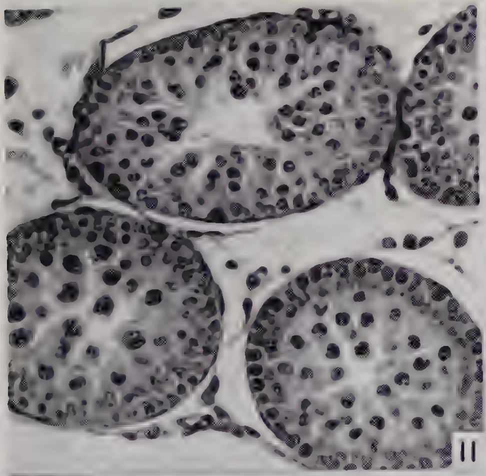
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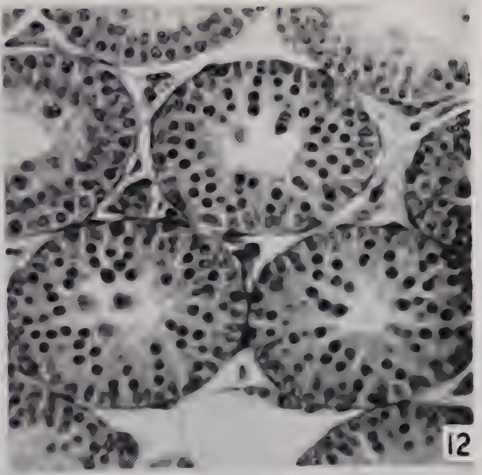
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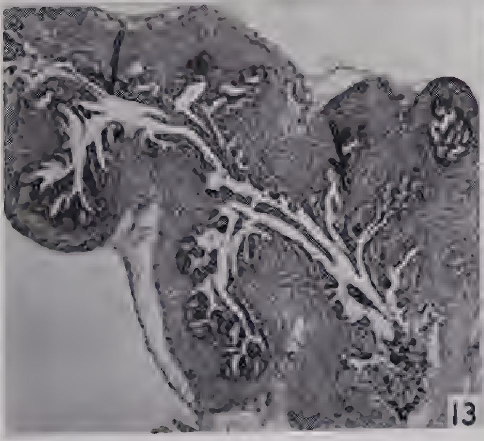
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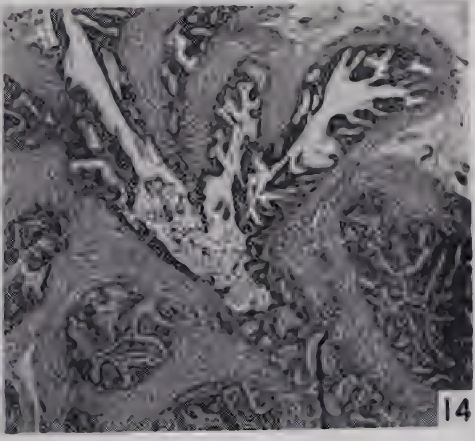
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MITOCHONDRIA IN THE INTESTINAL EPITHELIAL CELLS OF STARVED AND FED SALAMANDERS

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TWO PLATES (THIRTEEN FIGURES)

INTRODUCTION

Numerous investigations have been made on the relation of mitochondria to digestion and absorption. Animals from all the vertebrate classes have been utilized in these studies, but the results obtained are divergent and non-conclusive. The present study of the absorptive cells of the intestinal mucosa of Triturus pyrrhogaster attempts to throw more light on the mitochondrial changes by carefully controlling the nutritional conditions of the animals. At the same time the fat content of these cells was studied to determine whether it might be a factor involved in the changes.

MATERIALS AND METHODS

For this investigation ninety-one specimens of Triturus pyrrhogaster (Japanese salamander) were used. The animals were fed earthworms for 3 to 4 weeks before experimentation began. The well-nourished animals, killed by decapitation at intervals of ½ to 24 hours following the time of feeding, served as controls; twenty-six specimens were killed after starvation for 2, 3, 7, 10, 14, 30, 60, and 120 days. Sixteen animals starved for 2 weeks were re-fed for 4 days. These animals were killed at intervals of ½ to 24 hours after feeding. Of the animals starved for 1 month, 4 were re-fed once, 4 for 4 days, and 4 were fed until the weight lost during starvation had been regained. This required from 7 to 15 days.

Each animal, after being slightly dried with a paper towel, was weighed before being killed; also its total length and tail length were recorded. Animals which were starved were weighed at the beginning and at the end of the fast; those re-fed were weighed again at the time of killing. The general condition of the animal, the size of the fat bodies, and the degree to which food appeared digested were noted. The digestive tract was clipped out, its length measured, and pieces about 10 mm. long were immediately fixed in Regaud's solution (Lee, p. 308). For mitochondrial study two pieces of intestine were taken at 1.5 cm. and at 5 to 7.5 cm. posterior to the pylorus. An intermediate piece was preserved in Regaud's solution and stained with Sudan III to show fat. Tissue preserved for mitochondria was cleared in xylol, embedded in paraffin, and sectioned transversely at 3 or 4 microns. The material used for study of fat was embedded in 20% gelatin and sectioned at 12 microns with a freezing microtome. Regaud's or Heidenhain's iron hematoxylin stains were used for mitochondria.

For determination of the amount of water in the intestine, a piece was taken from the posterior end of the intestine, weighed in a dish on analytical balances, dried at 33° to 35° C., reweighed, and the percentage of water determined.

OBSERVATIONS

Well-nourished animals (controls). In the amphibian intestinal mucosa only two types of cells are recognizable, the absorptive and the goblet cells. The investigation is concerned chiefly with the absorptive cells. In the well-fed animals, regardless of the length of time after feeding, the mitochondrial picture of the epithelial cells shows no significant variation. The mitochondria are present as (1) rings, both round and oval; (2) granules of all types, many of which are connected to form a dumb-bell shaped rod; and (3) filaments and rods, some of which have thickened regions and occasional blebs (figs. 1 and 2). If only a few filamentous mitochondria are found present in the cells, the rods, spherules, rings and dumb-bell forms are numerous, and the short filaments present are curved, wavy, or beaded (fig. 1). If long filamentous mitochondria predominate there are fewer rings, dumb-bell forms and short filaments (fig. 2). The distribution of mitochondria is similar in the cells of all the well-fed animals. The cells may, for convenience in description, be divided into zones as follows: zone 1 extends from the intestinal lumen to about midway between the lumen and the nucleus; zone 2 extends from zone 1 to the nucleus; zone 3 is the region around the nucleus; and zone 4 the region below the nucleus (fig. 1). Zones 1 and 2 comprise the supra-nuclear region, zone 4 the sub-nuclear region.

Mitochondria are evenly distributed throughout zones 1 and 2, with occasionally a very slight aggregation in zone 1. The granular forms are usually more numerous in zone 1. Filaments are generally parallel to the long axis of the cell (fig. 2). In zone 4 the numerous mitochondria are mostly rods and granules with fewer rings and filaments than in the supra-nuclear cytoplasm.

The Sudan III tests for fat revealed either light orange droplets (fig. 3) or a diffuse yellow coloration of the cells. The quantity of fat is rather small in all these specimens. In some specimens the fat in concentrated in zones 2 and 4 (fig. 3), in the other specimens it is distributed throughout the cytoplasm of the absorptive cells of both the folds and the crypts of the intestinal wall (fig. 4). In addition to mitochondria and fat drops other granules occur almost universally in the supra-nuclear cytoplasm but are seldom observed in the sub-nuclear cytoplasm. These granules range in size from the diameter of the spherical mitochondria to that of large nucleoli; with iron hematoxylin they are stained black.

Starved animals. During starvation the body weight of an animal constantly decreases, while at the same time the per cent of water in the intestine increases; the fat bodies and liver decreases progressively in size; and the digestive tract decreases in length and diameter, with a corresponding reduction in the number of intestinal folds. The liver in addition to becoming thinner, grows much darker in color. The gall bladder is always filled with bile and often bile clots appear in the gut lumen. Histologically, there is an increase in the number of goblet cells in the mucosa of the intestine, a thinning and increased homogeneity of the striated border, and a loss of staining capacity by the epithelial cells. In animals starved for 4 months almost every second cell is a goblet cell.

The absorptive cells of animals starved for 2, 3, and 4 months are much smaller than the controls; the cytoplasm has shrunk relatively more in volume than has the nucleus (cf. figs. 2 and 5). The mitochondria have decreased somewhat in size; the filaments are thinner and fainter and the granules are smaller. Filaments with looped ends, blebs, or thickened places as well as ring-shaped mitochondria are infrequent. In zone 1 of the cytoplasm only granules occur, frequently associated in chains. The filaments are always short and occur in zones 2 and 3. Some of the filaments are beaded, others have a granular enlargement at each end. Scattered among the filaments are granules. The mitochondria of zones 2 and 3 are less compact than in zone 1. Zone 4 contains fewer mitochondria than in the controls (fig. 5). In the cells of animals starved 7 to 10 days filamentous mitochondria predominate (fig. 6); after 14 to 30 days granules predominate (fig. 7).

Some fat appears in the intestinal epithelial cells of practically all the starved animals. It is in a diffuse condition in the cells of all the mucosal crypts and folds. The dark granules previously described appear in the cells of nearly all of the starved animals. However, they are fewer than in the cells of the well-fed specimens. Animals starved for 1 to 4 months show fewer of these than animals starved for a shorter period of time.

Re-fed animals. Re-feeding of starved animals results in a restoration of the mitochondria to the normal (well-fed) condition. The granular mitochondria in the cells of the animals starved for 2 weeks and then fed transform into the varied forms characteristic of the controls. In like manner some of the granules, chains of granules, and thin threads characteristic of the animals starved for 1 month change into other forms when these animals are re-fed.

One re-feeding. Within 2 hours after a single re-feeding a change is evident in a few specimens in the morphology and especially in the distribution of the mitochondria. The normal distribution (numerous basally, evenly distributed supranuclearly) is restored, and ring-shaped mitochondria reappear, as well as short beaded filaments, and filaments with terminal loops (fig. 8). By 24 hours after the first feeding, animals starved for 2 weeks display many filaments in the supra-nuclear cytoplasm (fig. 9). The epithelial cells of most of the animals starved for 2 weeks and re-fed once contain more fat than the normal animals. This is generally distributed throughout the cytoplasm either as a diffuse dark yellow coloration, or as orange droplets (cf. fig. 10).

Re-feeding for 4 days. Animals starved for only 2 weeks and re-fed for 4 consecutive days show the normal mitochondrial picture similar to that found in control animals ½ to 4 hours after feeding (fig. 11). In general, body weight, cellular dimensions, fat content of the cells, and other gross and microscopic features have returned to normal.

The mitochondria in the epithelial cells of animals starved for 1 month and re-fed for 4 days present a similar picture to those of animals re-fed only once after the month's fast. However, the cells have increased somewhat in size. In some specimens the cytoplasm is vacuolated, and Sudan III tests reveal far more than the normal quantity of fat (cf. figs. 3 and 10). The cells of the apex of the folds are more heavily laden with fat droplets than the cells of the crypt regions.

Re-feeding until the pre-fast weight is restored. Animals starved for 2 weeks generally restore their pre-fast body weight after being fed for 4 consecutive days. Seven to 15 days of feeding were required to restore the body weight of animals starved for 1 month. A study of all these animals revealed that usually when the original body weight is restored other conditions have likewise returned to normal. The mitochondria in the epithelial absorptive cells are normally distributed and are of the normal shapes (cf. figs. 1 and 12); the fat content of the cells is normal in quantity and in distribution; the cellular dimensions are within the normal range; the cytoplasm has regained its staining capacity; the cellular membrane and striated border are of normal thickness and distinctness; and the percentage of water in the tissue has decreased. The digestive tract has expanded in diameter and in length, the number of intestinal folds has returned to normal (9 to 12), and the number of goblet cells is reduced to normal.

In some specimens the mitochondria in the cells on the apices of the folds respond to re-feeding more quickly than do those in the cells of the crypt zones. This is more evident in re-feeding after a month's fast than after only a 2-week fast.

Mitochondria in the posterior intestinal cells. The epithelial cells of the posterior sections of the intestine of well-fed animals show relatively more filaments, though fewer with looped ends, and fewer rings than the epithelium of the more anterior sections (cf. figs. 1 and 13). In the starved specimens the condition is the same as that described for the anterior sections, although the changes occur more slowly. Recovery during re-feeding is slower than in the anterior part of the intestine, although when the body weight has returned to normal the mitochondria are again like those in the cells of the well-fed specimens.

DISCUSSION

The morphology and distribution of mitochondria characteristic of active and inactive cells. Previous investigators of mitochondria in the intestinal epithelial cells have believed that the shape of these cytoplasmic inclusions is an index to the activity of the cell. Policard ('10), Champy ('11), Péterfi ('14), Corti ('12, '26), and Ryozo ('33) considered filaments characteristic of inactivity, rods and granules characteristic of activity. Shun ('20) concluded that the opposite was the case. Liu ('30) found supra-nuclearly thin threads, subnuclearly granules during inactivity; these enlarged and fragmented during digestion and absorption. Asher ('08), Demjanenko ('09) and Zillenberg-Paul ('09) observed granules in the cells under all conditions of activity, and noted a change in the number and distribution of these under different nutritional states. Eklöf ('14) found that during starvation the granular and rod-like mitochondria become aligned into filaments, which tended to converge to an angle just above the nucleus. In the cells of the well-nourished animals the mitochondria were more dispersed in the cytoplasm. He found the mitochondria more numerous and more distinct during activity than during starvation.

In contrast to the results of these earlier workers the present study reveals that during digestion and absorption all forms of mitochondria are present. They are uniformly distributed throughout the supra-nuclear cytoplasm and are typically more abundant in the sub-nuclear region. This study reveals that the correlation existing between mitochondria and digestive and absorptive activities is not merely one of mitochondrial shapes, but it involves changes in the relative number of forms, as well as the relative distribution of these forms in the cytoplasm. During inanition there is never at any one time the variety of form among the mitochondria which is common in well-fed animals (cf. figs. 1 and 2 with 5, 6, and 7). Filaments abound during the first 10 days of fasting (fig. 6), but 14 to 30 days of fasting produces granular forms (fig. 7). After 2 to 4 months of starvation both granules and filaments occur in characteristic positions in the cytoplasm (fig. 5). In contrast to the well-nourished animals, zone 1 in the cells of the specimen starved for 2 to 4 months contains only granules, more compactly placed than in the well-fed specimen; zones 2 and 3 show shorter filaments, many of which are either beaded or have terminal granular enlargements; and zone 4 contains fewer mitochondria. Starvation is accompanied by a reduction in the number of filaments with thickened places, and blebs and usually an elimination of the rings and terminally looped filaments.

Relationship of mitochondria to physiological activity of the cells. The intestinal mucosa of amphibians contains only goblet and columnar cells. It has been suggested (Holmes, '28; Noble, '31) that the columnar cells perform a dual role, secretion and absorption. The present study reveals that the mitochondria in these columnar cells of animals previously starved appear normal as early as 4 hours after re-feeding. It would seem probable that intestinal absorption does not begin so soon after a meal, since autopsy of the digestive tract in this study, as well as in that of McCurdy ('39), revealed that food remains in the stomach from 24 to 48 hours. Thus it appears probable that the immediate mitochondrial alteration in these intestinal cells may be initiated by enzyme elaboration. Cowdry ('18) considered thickened places and blebs on filamentous mitochondria as indicative of secretion. This study substantiates Cowdry's conclusions in that mitochondria of the type indicated are more abundant in the cells of the feeding animals than in the starved ones and are fewer in the cells of the lower end of the intestine where enzyme productivity is probably less pronounced. A further evidence for the fact that mitochondrial changes are correlated at least in part with enzyme secretory activity is the fact that in the posterior intestinal cells the changes in the mitochondria are slower and the differences are never so great as in the anterior intestinal cells where secretion is probably more pronounced.

If the secretive and absorptive functions of the intestine are performed by different cells of the intestinal mucosa the fact has not been clearly demonstrated. To what extent digestion and absorption are intracellular processes is unknown (Starling, '35; Best and Taylor, '39; Macleod, '41). Verzàr and McDougall ('36) and Macleod ('41, p. 986), state that fats and perhaps glucose and proteins are transformed intracellularly by the intestinal epithelial cells before they are passed into the circulating fluids of the body. It is believed (Champy, '11; Liu, '30) that mitochondria play an active role in these absorptive processes. The bipolarity in the arrangement of the mitochondria in cells is usually considered indicative of secretion in two directions (Cowdry, '18, '24, p. 318). In this study the partial loss of the bipolarity of the mitochondria in the cells of starved animals suggests a change in this two-fold secretion (cf. fig. 1 with figs. 5 and 7) and is an evidence that some of the mitochondrial modifications observed in these cells of normal animals are related to absorptive phenomena. Further evidence that mitochondria are associated with absorptive activity is that the restoration of the mitochondria to normal occurs first in the cells on the apices of the folds where absorption is believed to be most abundant. Sudan III tests show more fat in the apices of the folds than in the cells of the crypt regions.

McCurdy ('39) considered rings and terminally looped filaments in the liver cells as indicative of digestive and absorptive activity. This study of intestinal cells substantiates that claim. These forms are almost totally absent from the cells of the starved animals.

The fact that the response of the mitochondria to re-feeding is more clear-cut than is the response to fasting suggests that mitochondria are sensitive indices to the physiological state of the cells. Although in early starvation the amount of material in the digestive tract decreases, a small amount remains for some time. The depletion of the nutritive material from the cytoplasm is slow and gradual. The intake of food after the cells are practically depleted is a well defined event. The response likewise is clearly defined. Ingestion initiates throughout the digestive tract a series of changes (secretion of enzymes and hormones) concerned with digestion and absorption. Almost immediately upon ingestion the entire digestive tract seems to become sensitized. However, the posterior portion of the intestine becomes active slightly later than the anterior portion, which can be correlated with the probability that the lower portion of the intestine is more concerned with absorption than with the secretion of enzymes. Other portions of the digestive system become active later as indicated by a comparison of the intestine with the liver of

McCurdy's ('39) preparations from some of the same specimens used in the present study, which shows that resumption of the normal state in the liver occurs later than in the anterior intestine.

In the cells of well-fed animals a variety of mitochondrial forms exists at all times. Lewis and Lewis ('15) found this condition to be characteristic of physiologically active cells. The series of changes observed at intervals from 2 to 120 days of starvation indicates that despite the absence of activity of the organ in which the cells occur, the mitochondria still are capable of undergoing to a limited extent the same transformations noted in the controls. If the size, distribution, and greater variability in shape of the mitochondria in the cells of the controls are correctly correlated with the greater physiological activity of the intestine in these animals, then the reduction in size, and the relative stability, as well as the change in distribution of mitochondria in the intestinal cells in animals starved for some length of time furnishes an index of their lowered physiological activity. The rapidity with which the mitochondria return to normal when the starved animals are re-fed shows the extreme sensitivity of these bodies when the organ again becomes active.

SUMMARY AND CONCLUSIONS

1. During digestion and absorption all forms of mitochondria are present in the intestinal epithelial cells. They are evenly distributed in the supra-nuclear cytoplasm, but are more abundant in the sub-nuclear zone.

2. During starvation the variation in the form of mitochondria decreases, their size diminishes, and their distribution in the cells is altered.

3. Re-feeding reverses the inanition changes and restores the mitochondria in size, distribution, and morphology to those characteristic of the well-nourished state.

4. Mitochondria in the form of rings and looped-end filaments, as well as filaments with blebs and characteristic

elongated enlargements, seem to be associated with digestive and absorptive activity of the cells.

5. Mitochondria are believed to be sensitive indices to the physiological state of the cells.

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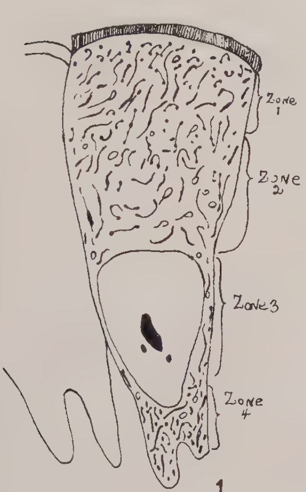
PLATE 1

EXPLANATION OF PLATES

All figures were made with the aid of a camera lucida at a magnification of approximately 750 times.

EXPLANATION OF FIGURES

1 Absorptive cell from control animal killed 2 hours after feeding.
2 Absorptive cell from control animal killed 6 hours after feeding.
3 Absorptive cell from control animal showing fat droplets.
4 Absorptive cell from control animal showing fat throughout.
5 Absorptive cell from animal starved for 3 months.
6 Absorptive cell from animal starved for 1 week.


Zone 1
Zone 2
Zone 3
Zone 4

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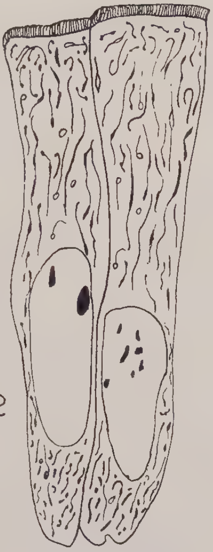

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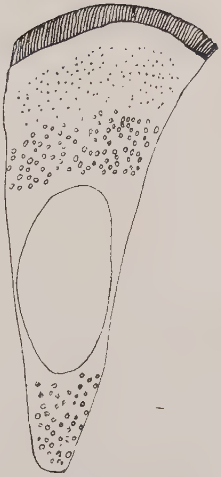

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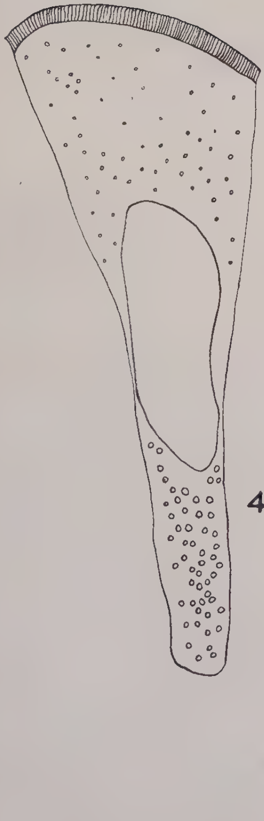

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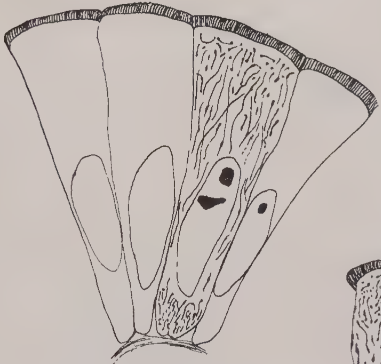

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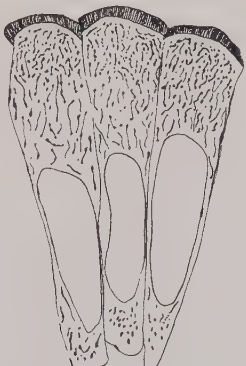

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PLATE 2

EXPLANATION OF FIGURES

7 Absorptive cell from animal starved for 2 weeks.

8 Absorptive cell from animal starved for 2 weeks, and killed 2 hours after being re-fed only once.

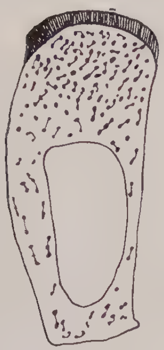
9 Absorptive cell from animal starved for 2 weeks and re-fed once; killed 24 hours after feeding.

10 Fat droplets in an animal starved for 1 month, then re-fed for 4 days. (Killed 6 hours after feeding.)

11 Absorptive cell from another animal re-fed for 4 days after a fast of 2 weeks, and killed 12 hours after feeding.

12 Absorptive cell from animal re-fed after a month's fast, until the original body weight was restored. (Seven consecutive days.)

13 Posterior intestinal cell from a control animal killed 12 hours after feeding.



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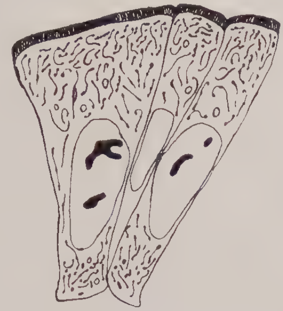

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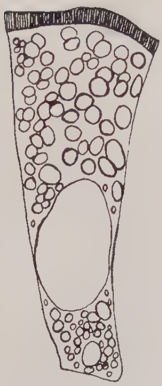

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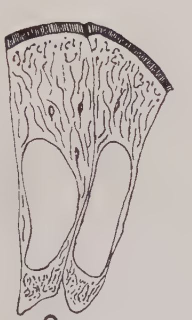

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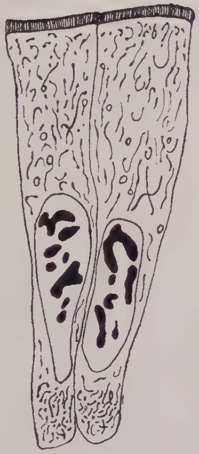

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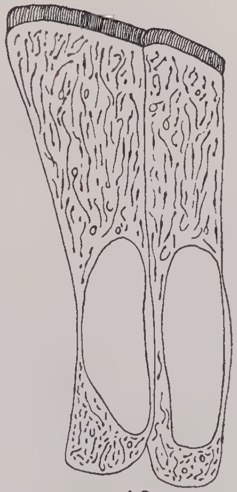

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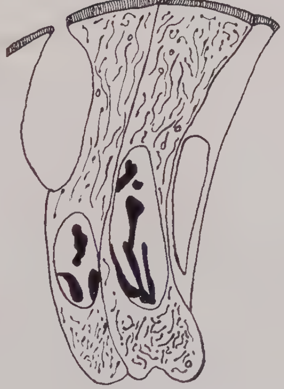

12

RELATION OF INTERSTITIAL CELL HYPERPLASIA TO SECRETION OF MALE HORMONE IN THE SPARROW[1]

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TWO PLATES (THIRTEEN FIGURES)

Although many suggestive data have been accumulated, conclusive evidence that the interstitial cells of the testis represent the sole source of the hormone produced by this organ is lacking. Most of the evidence in favor of the interstitial cell origin of the hormone has been collected from studies of mammalian material (Rasmussen, '32; Moore, '32). On the other hand, careful study of the testes of many species of birds has failed to reveal any correlation between histological or cytological characteristics of the intertubular elements and masculinization (Boring and Pearl, '17; Bissonnette and Chapnick, '30; Goodale, '19; Loisel, '03; Oslund, '28; Stieve, '19; Nonidez, '24 a and b, and Watson, '19). Cells of the avian testis which are referred to as "interstitial cells" by many authors are more than likely merely cellular elements of the intertubular connective tissue.

It seemed possible that if true interstitial cell hyperplasia could be induced experimentally in the testis of an avian species which possesses negligible numbers of Leydig cells,

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and if the appearance of such cells was found to be correlated with androgen secretion, more information would be gained relative to the general problem of the testicular interstitial cell. This method has proved feasible inasmuch as it was found that administration of certain gonadotropins to the English sparrow resulted in the appearance of Leydig cells which could be identified without question for the first time. In previous studies on the English sparrow the authors were never able to positively identify any cells as true Leydig cells although material representing all phases of testicular activity were studied.

MATERIAL AND METHODS

The sparrows used in these experiments were trapped in nature. Normal animals were collected throughout the year and autopsied immediately. Others were trapped in late summer and, after acclimatization to the laboratory, were divided into five groups and treated as outlined below. One-day-old White Leghorn chicks purchased at a local hatchery comprised a sixth group of experimental birds.

I. Daily injections of PMS for relatively short periods of time. Twenty-five juvenal males were injected daily with 12.5 r.u. of pregnant mare serum [2] (PMS) for from 4 to 70 days during the fall months (September to November), most of the birds being sacrificed between the fifth and fifteenth day after treatment was begun. Sixteen juvenal males received only 0.5 r.u. of PMS daily during December and January for from 7 to 33 days.

II. Daily injections of PMS for long periods of time. Six males were injected with 6.25 r.u. of PMS daily for 200 or more days beginning in September. Six females started at the same time received 12.5 r.u. of PMS for from 98 to 148 days.

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[2] Pregnant mare serum (Gonadin), which for the sake of brevity will be referred to as PMS, was kindly furnished by the Cutter Laboratories through the courtesy of Drs. D. H. Wonder and C. Parham. The dosage is expressed in terms of the Cole-Saunders rat unit, which is biologically equivalent to two international units.

III. Regression of interstitial cells following treatment with PMS. In order to study the history of the interstitial cells during regression of a testis previously stimulated to full activity by pregnant mare serum, twelve male sparrows were injected daily with 12.5 r.u. of PMS for 10 to 21 days and sacrificed 2, 5, 9, 10, 11, 15, and 20 days after treatment had ceased.

IV. Injections with anterior pituitary hormones.[3] Ten juvenal males received 2 mg. daily of an aqueous suspension of sheep whole pituitary powder for from 27 to 36 days. Five juvenal males were given 1 r.u. daily of a partially purified preparation of pig pituitary powder, and three were injected daily for from 8 to 12 days with 10 r.u. of the same material.

V. Increased daily light ration. A group of male sparrows was subjected to 8 hours of added light daily during December and January. Birds were killed at weekly intervals.

VI. Stimulation of interstitial cells by PMS in young chicks. Eleven male and two female 1-day-old White Leghorn chicks received 12.5 r.u. of PMS for 5 days and thereafter, for 7 to 30 days, received 25 r.u. of PMS. Thirteen untreated males and one untreated female served as controls.

All injections were made into the breast muscles. When the males were autopsied one testis was fixed in Bouin's fluid, and was subsequently sectioned and stained with hematoxylin and eosin. The other testis was impregnated with osmic acid according to the method of Gersh ('39). Ovarian material was treated similarly, one-half of the ovary being fixed in Bouin's fluid, the other half being osmicated.

The level of androgenic secretion was estimated on the basis of bill pigmentation in the case of the sparrow and the weight of the comb in the case of the chick. The fresh weights of these organs were obtained by means of a torsion balance. Measurements of testes were made with calipers previous to fixation. The birds were fed a mixture of chick growing mash and cracked grain and given fresh water twice daily. They were kept in wire cages which were 3 × 3 × 4 feet in dimension, each cage housing about twenty birds.

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[3] TheAcetone dried sheep whole pituitary powder was purchased from Wilson Laboratories and the partially purified pig pituitary powder was kindly furnished by Dr. R. C. Bruner of Chappel Laboratories.

OBSERVATIONS

In untreated birds there was no time during the normal testicular cycle when recognizable Leydig cells could be definitely identified. In spite of this absence of cells which are supposed to secrete the androgenic hormone, the bill of the normal male sparrow in nature darkens appreciably as early as January, when the spermatogonia are the only germinal cells undergoing mitotic division. Such divisions of spermatogonia are the first sign of imminent testicular enlargement. In March, when the bills of all male sparrows are jet black, the intertubular tissue is merely a loose connective tissue (figs. 3 and 4) comparable to the loose areolar tissue which might be found beneath the skin. This tissue becomes stretched and condensed as the rapidly growing tubules expand and comes to form a very thin capsule around the fully matured tubules, with only a small area of fibroblasts and blood vascular elements in the angles between them. The germinal epithelium is extremely active at this time, and the males exhibit mating behavior, further indicating the presence of androgen.

I. As early as 3 or 4 days after the first daily injection of 12.5 r.u. of PMS the yellow bill of the male sparrow showed a green tinge. This coloration was the first external manifestation of androgenic secretion (Pfeiffer and Kirschbaum, '41). The testes of such birds were only slightly enlarged (2 mm. × 2 mm.), most of the hypertrophy resulting from an increase in the amount of intertubular tissue. In most cases there was no mitotic activity in the germinal epithelium (fig. 7). The cytology of the interstitial tissue was markedly altered. The intertubular spaces, which in the normal resting testis were occupied by loose connective tissue containing lymphocytes (fig. 1), were now filled with large "epithelioid cells" of the Leydig type (fig. 9). When stained with hematoxylin and eosin these cells showed a fine eosinophilic granulation in the cytoplasm. This material was presumably the same as that which became impregnated with osmic acid (fig. 12). The Leydig cells were the only cells of the testis which showed an affinity for osmic acid.

Occasional mitotic figures could be observed in the interstitial cells. The increase in the number of interstitial cells resulted, primarily, however, from transformation of fibroblasts into these elements (fig. 11). Often small scattered groups of lymphocytes were the only elements of the intertubular areolar tissue of the resting testis which remained unchanged.

When a very low dose of PMS (0.5 r.u. daily) was injected into juvenal males, the testicular response differed from that shown when the higher dose (12.5 r.u.) was administered. The primary response to the lower dose was in the germinal epithelium, with little or no interstitial cell response. It was evident that these gonads secreted appreciable amounts of male sex hormone since the bills darkened considerably during the first week of treatment. Gonads stimulated by 0.5 r.u. of PMS daily for 7 days were similar histologically to the January and February testes of birds living free in nature.

II. Upon continued stimulation with daily injections of 12.5 r.u. of PMS spermatogenesis went to completion. As the seminiferous tubules enlarged the interstitial cells were squeezed into the now greatly narrowed intertubular spaces but continued to maintain their identity. Although there were now fewer Leydig cells per unit volume of testis, the absolute number of these cells was not necessarily decreased.

Long term treatment with PMS (200 or more daily injections of 12.5 r.u.) produced no greater hypertrophy of the interstitial cells. In fact these elements were less apparent in animals receiving this treatment than where the germinal epithelium was only partially stimulated. With long periods of treatment the seminiferous tubules became markedly dilated and contained great numbers of free spermatozoa. Many areas showed signs of becoming depleted of germinal cells, the germinal epithelium being thinner than usually seen in a spermatogenically active testis. The interstitial cells were squeezed into the interstices between the dilated tubules.

The ovaries of females injected with PMS for a long period of time produced appreciable quantities of androgen as judged by bill pigmentation (see Pfeiffer and Kirschbaum, '41). However, neither the interfollicular tissue nor any cellular elements of the Graafian follicles contained the osmiophilic granules characteristic of Leydig cells. The so-called "luteal-like" (interstitial) cells were not prominent in the medullary portion of the ovary. No definite correlation has as yet been found between any specific cell type and ovarian androgenic secretion.

III. When the testes were allowed to regress following preliminary treatment with PMS, the Leydig cells reverted to connective tissue cells of the type found in the inactive gonad. Some variation in the rate of regression of both the tubules and the interstitial cells occurred, but by about 9 days after cessation of treatment the tubular volume had decreased greatly, and all stages from spermatocytes on were sloughing. The interstitial cells had become smaller and the fine eosinophilic granulation in the cytoplasm had been lost. In certain areas these cells appeared to be transforming into lymphoid cells, possibly as a step in their dedifferentiation to mesenchymal cells. For a time lymphoid tissue appeared to be present in appreciably increased amounts, but gradually the proportion of lymphocytes to connective tissue cells was restored to that of the normal inactive testis. There was no evidence that the Leydig cells underwent complete degeneration and were removed by phagocytes.

IV. Following injections of anterior pituitary materials the germinal epithelium showed marked development (figs. 5 and 6), but in most cases, except for the presence of a few Leydig cells, the intertubular tissue (figs. 5 and 6) resembled that found in the testis during the normal seasonal enlargement (figs. 2 to 4). This was particularly true with the partially purified pig pituitary preparations. However, occasionally isolated nests of true interstitial cells were found, especially when higher doses were used, and in one case an extreme increase in these cells occurred (fig. 8), resembling the condition found following treatment with large doses of PMS. Except in the few cases where numerous Leydig cells appeared, the testes of birds receiving anterior pituitary extracts had to attain a much greater size before sufficient amounts of androgen were produced to cause the bill to darken than did the testes of birds free in nature or of birds stimulated with large doses of PMS. In this respect the effects of the anterior pituitary extracts resembled those produced by exposing the birds to increased daily light ration (section V).

V. The testes of most of the sparrows which received 8 hours of added light daily during December and January were stimulated within 6 weeks to full spermatogenic activity. Unlike the condition encountered in nature the testes attained an appreciable size before a detectable amount of male hormone was produced. However, the development of the testis was histologically identical with that occurring in nature, and there was no evidence of the formation of true Leydig cells at any time during the period of stimulation.

VI. Administration of relatively large doses of PMS to chicks for 12 to 35 days after hatching stimulated the formation of true interstitial cells in the testes. These cells were formed from the fibroblastic cells of the intertubular tissue as described in the sparrow, but the process seemed to occur more slowly than in the sparrow, and all stages in the transformation were readily seen (fig. 10). The processes of the fibroblastic cells were withdrawn and the cells and their nuclei became ovoid to round in shape. Meanwhile, fine eosinophilic granules began to appear. The newly formed interstitial cells were smaller than the mature cells and had densely granular cytoplasm. As the cells grew larger and became secretory their cytoplasm became lighter and, following Bouin's fixation and hematoxylin and eosin staining, were observed to be filled with fine vacuoles. The later stages of maturity usually did not occur in these young chicks. The testis enlarged only slightly and there was only a slight increase in male hormone output.

In female chicks interstitial cells, which have been described as luteal-like cells, were rather abundant in the immature ovary. These cells were stimulated by PMS treatment and in at least one case such stimulation was correlated with an unusually large comb, indicating androgen production.

DISCUSSION

The mechanism of androgenic secretion in the sparrow, at least, seems to be problematical. Although true Leydig cells were not distinguishable in the normal sparrow testis, their appearance could be stimulated by the administration of gonadotropic materials. Since in certain cases such cells were caused to appear before the germinal epithelium of the inactive testis was stimulated to spermatogenic activity, and since concommitant with the appearance of the interstitial cells, pigment was deposited in the bill, a definite indication of androgenic secretion (Keck, '33; Witschi and Keck, '35; Pfeiffer and Kirschbaum, '41; Kirschbaum and Pfeiffer, '41), it seems probable that under these experimental conditions the male hormone was produced by the Leydig cells. However, the absence of Leydig cells when testicular development occurred during the normal sexual cycle (figs. 2 to 4) indicates that under the influence of the bird's own hypophysis the germinal epithelium must control the production of androgen.

Breneman's ('35) observations favor the germinal epithelium as the source of androgenic hormone in the cockerel. When immature birds were treated with FSH, only tubular development resulted, and comb growth was marked. Under the influence of LH the interstitial cells were stimulated, and there was no appreciable increase in the size of the comb.

In the present study Leydig cells were readily formed in young chicks receiving PMS treatment, but the amount of male hormone secreted was not greatly increased. The interstitial cells, however, did not take on the somewhat vacuolated appearance of the actively secreting cells, suggesting the possibility that two factors are necessary to bring about androgen production by interstitial cells, one causing formation and the other the secretory activity of these cells. The latter may be present in appreciably smaller amounts in PMS or, at least, may be less effective under certain conditions (Pfeiffer and Hooker, '42). The gonadotropic preparations used in these experiments both stimulated the germinal epithelium and caused the appearance of Leydig cells. However, with the larger doses of PMS the Leydig cell response occurred first. With minimal doses both responses occurred simultaneously, but the germinal epithelium differentiated much more rapidly than did the Leydig cells. The latter reaction also followed administration of sheep whole pituitary powder and partially purified pig pituitary powder. Except for the presence of a few Leydig cells, the response of the testis to the pig pituitary powder was like that which occurs under the influence of the bird's own hypophysis, furnishing further support for the generally accepted opinion that the two elements of the testis are under different endocrine control. However, the present experiments do not rule out the possibility that a higher level of gonadotropic hormone is required to bring about the Leydig cell response than necessary for stimulation of the germinal epithelium. That the presence or absence of interstitial cells could be due to faulty technique (Benoit, '22) is untenable since the Leydig cells described here are readily identifiable by ordinary histological methods.

Testicular interstitial cells have been described by many authors for various avian species, e.g., cockerel (Benoit, '22; Reeves, '15) junco (Rowan, '29), sparrow (Pellegrini, '25), crow (Stieve, '19), duck (Benoit, '36) and starling (Bullough, '42). However, these cells could not be demonstrated in the cockerel by Boring and Pearl ('17) and Goodale ('19), in the untreated sparrow by the present authors, nor in adult birds of other species, e.g., the starling (Bissonnette and Chapnick, '30), the crow (Stieve, '19), and the greenfinch (Watson, '19). Nonidez ('24 a and b) found very few in the cockerel. Benoit

('22) has emphasized the fact that special techniques must be used to demonstrate these cells. The suspicion is that many of the investigators reporting interstitial cells in the bird have very carefully described the few they found and were influenced by the preconceived idea that interstitial cells should be present in spermatogenically active testes, regardless of vertebrate class or species. It is also apparent that many authors have included as interstitial cells fibroblasts and histiocytes of the intertubular connective tissue.

The interstitial cells which appeared in the testes of both the sparrow and the chick under the influence of gonadotropic hormone arose from fibroblastic elements (figs. 10, 11 and 13). When such testes regressed after cessation of treatment with pregnant mare serum, the Leydig cells probably reverted to fibroblasts. It is possible that the lymphocyte represents an intermediate cell type in this reversible transformation, since at certain stages in regression the Leydig cells appeared to be transforming into lymphocytes. At least, the amount of intertubular lymphoid tissue was increased. This increase was not permanent, and the ratio of lymphoid to connective tissue elements rather quickly reverted to that found in the quiescent testis.

The very distinct variation in the size of sparrow testes, attained under normal and the various experimental conditions, before they produced enough androgen to blacken the bill obviously cannot be explained on the basis of the presence or absence of either interstitial cells or activated germinal epithelium. In birds free in nature, where the testes contained no interstitial cells, the bill blackened when the testes were still very small. A similar bill reaction with testes almost as small occurred in birds receiving large doses of PMS, and in this instance the interstitial cells were the only elements stimulated. In nature male sparrows were found to have black bills when their testes had barely begun to enlarge while testes of comparable size in birds on increased daily light ration were not effective in darkening the bill. This does not necessarily indicate a greater androgenic output by the former since the birds living free in nature might respond more readily to minimal amounts of hormone. Moreover, the gradual stimulation of the testes which one might assume to occur in nature might be much more effective in bringing about the secretory phase of the precursor cells.

SUMMARY

1. Although in the male sparrow the production of androgen during the normal sexual cycle and following stimulation of the testis by increased daily light ration was indicated by the deposition of melanin in the bill, Leydig cells were not found. Apparently under these conditions androgen originates in the germinal epithelium.

2. When the testes were stimulated by injections of large amounts of PMS (12.5 r.u. daily) or hypophyseal extracts, Leydig cells appeared between the testicular tubules. In a few cases such hyperplasia occurred, and androgen was secreted, in the absence of a spermatogenically active germinal epithelium, suggesting that under these conditions Leydig cells were the source of the androgen. On the other hand, with a lower daily dose of PMS (0.5 r.u.) the germinal epithelium was preferentially stimulated with little or no Leydig cell development, and here, also, the level of androgen secretion was high.

3. Under the influence of injected gonadotropic hormone the connective tissue cells of the intertubular areas transformed into Leydig cells. After the cessation of the gonadotropic stimulation the interstitial cells apparently reverted to fibroblasts. However, there was some indication that they might transform into lymphocytes as an intermediate stage in this regression.

4. No cells comparable to testicular Leydig cells were found in sparrow ovaries which were secreting androgen during the normal cycle or following stimulation with PMS.

5. Injection of PMS into White Leghorn chicks for 12 to 35 days after hatching caused development of the classical Leydig cells in the testes. However, the production of androgen

was only moderately accelerated. Treatment with PMS also stimulated the production of interstitial ("luteal-like") cells in the chick ovary. These cells may be associated with androgen production.

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PLATE 1

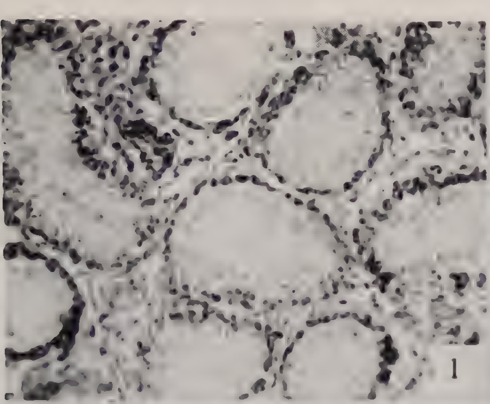
EXPLANATION OF FIGURES

All figures are photomicrographs of sections of testes stained with hematoxylin and eosin.

1 The sparrow testis during the non-breeding season. Tubules contain only spermatogonia and Sertoli cells, while the intertubular tissue is composed of loose connective tissue, lymphocytes and blood vascular elements. × 300.

2, 3 and 4 Stages in the normal development of the sparrow testis. Intertubular tissue remains unchanged except that it is stretched and the space between any set of tubules is reduced as spermatagenesis goes to completion. × 300.

5 and 6 Development of the sparrow testis following injection of 2 mg. of sheep pituitary powder daily for 25 and 30 days respectively. These stages are essentially the same as those occurring in the sparrow free in nature. (Compare with figs. 2, 3 and 4.) × 300.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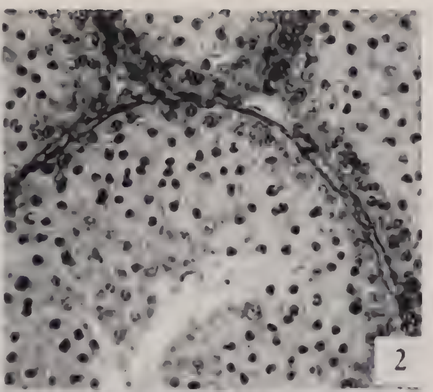
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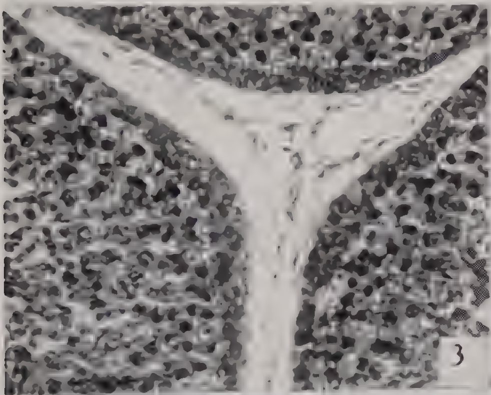
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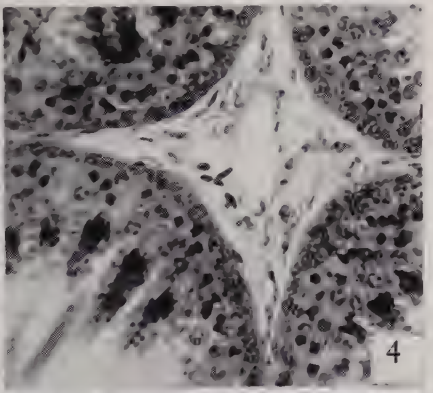
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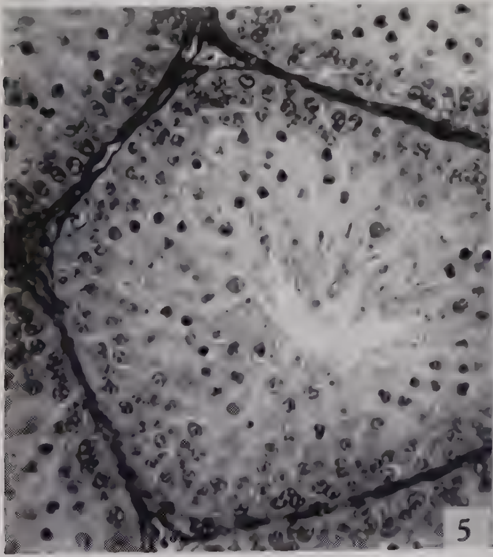
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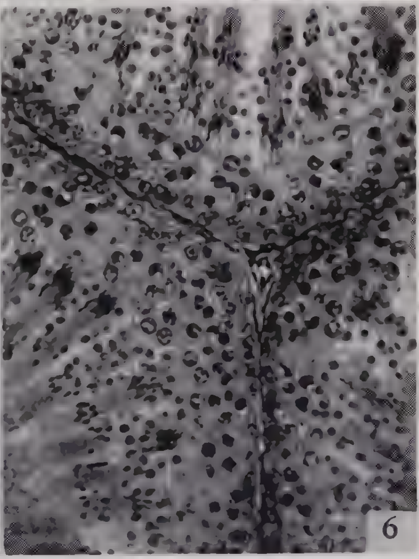
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PLATE 2

EXPLANATION OF FIGURES

All figures are photomicrographs of sections of testes which, with the exception of figure 12 are stained with hematoxylin and eosin.

7 Section of sparrow testis after 10 days of treatment with 12.5 r.u. of PMS daily. Note the great hypertrophy of true Leydig cells without any appreciable spermatogenic activity. There was very little increase in testis volume. × 300.

8 Section from the testis of a sparrow which had received 10 r.u. of partially purified pig pituitary powder for 10 days. This was the only animal treated with anterior pituitary powder which showed only Leydig cell development. The others showed predominately spermatogenic activity with some Leydig cell development. × 300.

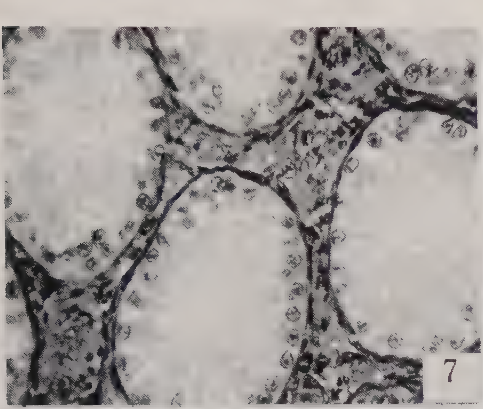
9 A large nest of true interstitial cells (Leydig cells) in an intertubular space, following 15 days of treatment with 12.5 r.u. of PMS daily. × 600.

10 Transformation of fibroblasts into true Leydig cells in the testis of a chick treated with 12.5 r.u. of PMS for the first 5 days after hatching and then with 25 r.u. daily for 7 days more. × 600.

11 Transformation of fibroblasts into interstitial cells in the testis of a sparrow which had received 12.5 r.u. of PMS daily for 5 days. × 600.

12 An intertubular area from the testis of a sparrow which had received 12.5 r.u. of PMS daily for 15 days. Note the osmiophilic granules in the interstitial cells, indicating that these cells are true Leydig cells. Impregnated with osmic acid. × 1000.

13 Testis of a chick treated with 12.5 r.u. of PMS for the first 5 days after hatching and then with 25 r.u. for 7 days more. Note the typical formation of Leydig cells without stimulation of the germinal epithelium. × 300.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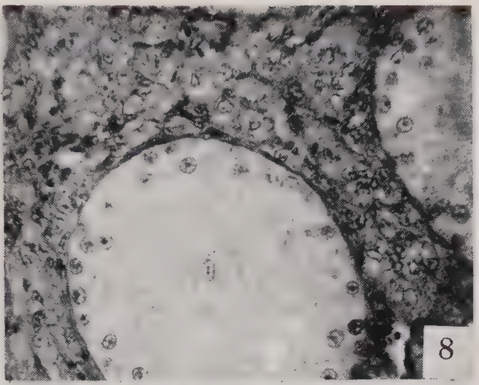
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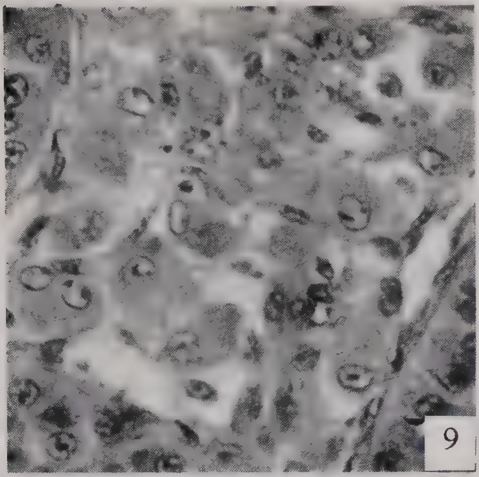
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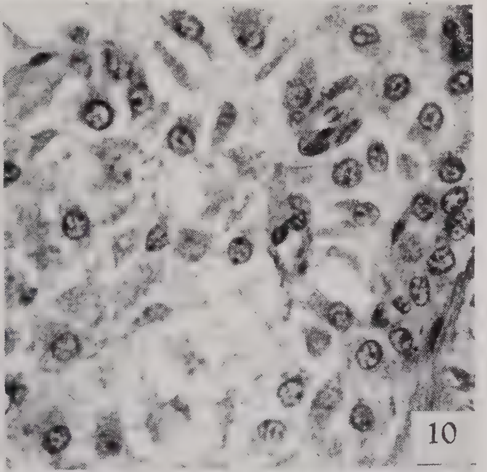
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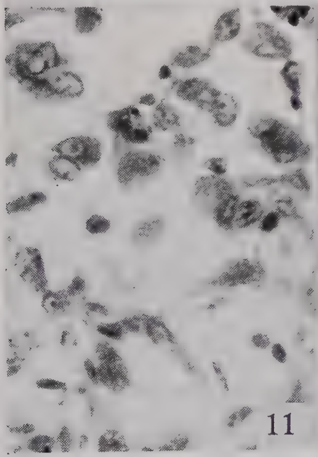
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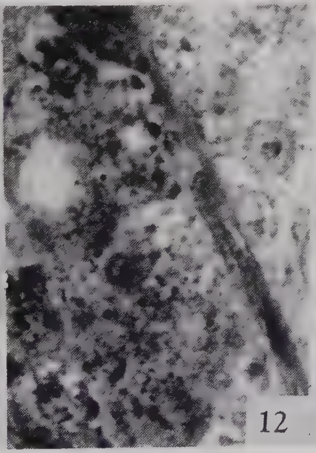
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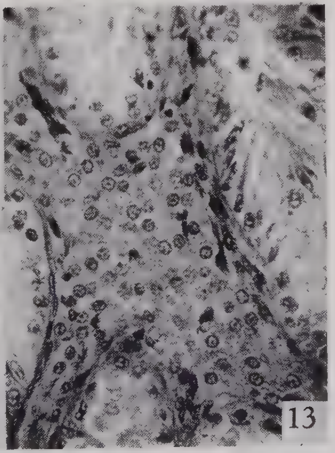
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THYMUS IV IN THE PIG

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ONE TEXT FIGURE AND TWO PLATES (TEN FIGURES)

INTRODUCTION

In an earlier study of complex IV in the pig (Godwin, '40 a) it was found that the ultimobranchial body early lost its connection with the pharynx and with the other components of complex IV and became buried in the thyroid gland. It was determined that the fourth pouch was quite variable in its formation and was frequently entirely lacking on one or both sides. The parathyroid IV was found frequently to break up into upper and lower components located at or near the point of origin on the dorsal and lateral surfaces of the laryngopharynx and near the posterior and caudal angle of the thyroid cartilage respectively. Fragments of pouch IV exclusive of parathyroid IV were followed with care to determine their exact location and fate. These fragments were found to form incipient thymus. No definitive thymus however was found at that time. Since definitive thymus IV is known to be tardy in formation in other forms a study of older stages was begun. A preliminary report of this study (Godwin, '40 b) showed that a definitive thymus IV was formed from pouch IV in the pig. The present article deals with the origin of definitive thymus IV from pouch IV and with problems of the thymus.

The question of whether or not thymus IV really comes from pouch IV, and pouch IV only, has been particularly difficult to solve. However, recent evidence has served to support the view that thymus IV arises from pouch IV exclusively and thus replaces the perhaps dominant view that any part of complex IV could and, on occasion, did form thymus IV. Godwin ('39) believed that thymus developed from pouch IV in the dog. Kingsbury ('41) finds a thymus formed from the pouch IV in the opossum. Van Dyke ('41) gives strong evidence that pouch IV forms thymus IV in man but does not feel, apparently, that his evidence warrants a definite conclusion on this point. The evidence will now be presented showing that in the pig thymus IV arises from pouch IV material.

MATERIALS AND METHODS

The observations of primary concern in this article were made upon serial sections of the laryngeal region of twelve near-term fetuses. Serial section of a wide area is imperative since variations in the development of the pouch make prediction of its exact location impossible. In addition serial sections of the laryngeal region of fourteen pigs of 100 mm. to 165 mm. crown rump length, which had been used in an earlier investigation, were frequently studied for purposes of orientation and comparison.

OBSERVATIONS

The specimens to be described are all near-term fetuses, the exact age of which is not known. This is of little or no importance because the variability found in specimens of the same age is so great that even considerable variation in age would make little difference for the purposes of this study. The material will be treated according to stage of development without regard to size or age.

In earlier specimens of 100 to 165 mm. crown rump length prethymus was found but no definitive thymus (Godwin, '40 a). In the near-term material prethymus is found at times continous with or closely associated with abortive, abnormal or definitive thymus. It is these stages which clearly show that the prethymus material actually becomes definitive thymus. Figure 2 shows a strand of epithelial cells continuous with a clump of the same type of cells which, however, have many thymocytes[1] enmeshed between them. Wherever the thymocytes are present the strand is much thicker than where they are absent. The question of the origin of the thymocytes presents itself at all times but it must be confessed that no satisfactory solution can yet be given. The question will however receive attention at appropriate points in the description and discussion. The epithelial tissue and associated thymocytes do not have the necessary characteristics for definitive thymus. There is no cortex-medulla and no Hassall's corpuscles. Thymocytes are present in great numbers in only part of the material and only in some regions may the beginning of lobulation, so characteristic of the thymus, be seen. The material is located in the subepithelial connective tissue of the dorso-lateral part of the laryngopharynx about midway between the level of the cephalic edge of the thyroid cartilage and the hyoid bone. Figure 1, figure 10 and reference to figure 1 in Godwin ('40 a), will make the location of this and material subsequently described easier to understand.

A small isolated fragment of pouch IV is shown in figure 3 beside material that has already undergone thymic transformation. It has been noted earlier (Godwin, '40) that pouch IV, when present may break into several pieces and become widely distributed during the shifting of parts in development. This epithelial fragment would soon have become typical thymus. It is made up, as is the material in figure 2, of large epithelioid cells with a large spheroid nucleus which has fine chromatin particles fairly evenly distributed in it. The cytoplasm has a slight tendency to take basic dyes. The cells are frequently in balls and clusters. The general picture is similar to the condition of prethymus found in the dog (Godwin, '39). A number of lymphocytes (thymocytes?) are present in the surrounding connective tissue. Whether or not they are entering or leaving the thymus could not be determined.

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[1] The term thymocyte is used in this article to designate the small deeply staining cells commonly found in great numbers in the cortex of the thymus without any implication as to their origin.

A definitive thymus IV is shown in figure 4. Here the cortex and medulla and lobulation of the thymus can be readily distinguished. Typical Hassall's corpuscles are also present in this thymus. The thymus lies in a gap in the pharyngeal musculature. The arrangement of the strands of connective tissue indicate that the thymus has expanded by pushing through the muscle layer laterally. Near the point on the pharyngeal epithelium where the fourth pouch becomes detached from the pharynx (see arrow) there is a lobe of prethymus tissue. This fragment is similar to other prethymus fragments and, in addition has a small remnant of the pharyngobranchial duct still attached to the pharyngeal epithelium. This prethymus material is located adjacent to the region of the laryngopharynx from which the complex IV becomes detached either wholly or in part.

Figure 5 illustrates a small atypical thymus near a prethymus region in the epithelial wall of the laryngopharynx. The surface region of this prethymus has a great number of thymocytes between the epithelial cells and can be regarded as having undergone a partial thymic transformation. A small cystic remnant of the pharyngobranchial duct is present on the deep surface of the material. Two cases of prethymus material in the pharyngeal epithelium have been previously noted (Godwin, '40 a).

A succession of steps showing the incomplete transformation of surface prethymus regions is shown in figures 6 to 9. This incomplete development results from the fact that frequently a part of pouch IV remains in the epithelium of the pharynx (Godwin, '40 a). In every instance the thymic region is sharply set off from the adjacent epithelium. No indication of a transition from one region into the other was observed. The thymocytes are found in greatest numbers near the surface. The epithelial cells loosen and become somewhat basophilic in their staining reaction first and then thymocytes begin to appear between the epithelial cells. The thymocytes become so dense that it is difficult to distinguish the epithelial cells (fig. 9). Usually a few cells which are exactly like the thymocytes in appearance are present in the adjacent connective tissue. The source of the thymocytes could not be determined. It does not appear that the thymocytes pass into the pharyngeal cavity in any great numbers. The surface layer of epithelial cells is usually quite complete. The epithelial cells are of the same kind as those of the previously described prethymus fragments. These surface thymic regions are always small and located at the same point on the dorsolateral surface of the laryngopharyngeal wall. Comparison of these thymic regions with developing tonsilar regions shows that these processes are not the same.

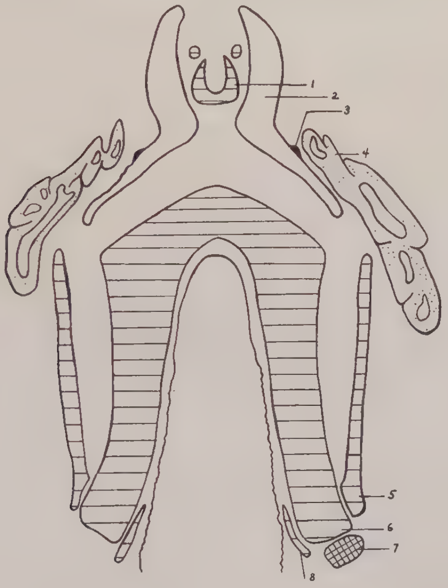

Fig. 1 This diagram was made from a series of projections and represents the conditions in the specimen shown in figure 10. This is a frontal section through the laryngeal region of a near-term fetal pig. (1) cartilage of the epiglottis, (2) cavity of the laryngopharynx, (3) intraepithelial thymic material, (4) thymus IV, (5) thyroid cartilage, (6) cricoid cartilage, (7) a fragment of the ultimobranchial body, (8) the first tracheal ring.

Figure 10 is a frontal section of the larynx region and shows a thymus IV on both sides of the pharynx. This specimen had the largest thymi found in this study. Figure 1 is a reconstruction of the actual conditions found in this specimen. The thymus material is typical in that it has lobules cortex-medulla, thymocytes and Hassall's corpuscles. The thymus in both cases almost touches the epithelium at the point of earlier detachment from the pharynx. On both sides the lower end extends caudad a short distance beyond the cephalic edge of the thyroid cartilage. There is a patch of surface prethymus epithelium on both sides indicating the exact point of detachment of that part of the fourth pouch which has subsequently formed thymus.

DISCUSSION

The exact fate of complex IV (ultimobranchial body and pouch IV with its parathyroid component) has given rise to many papers and much discussion. The real difficulty is brought about by the fact that in many common animals complex IV is associated or fused with the thyroid gland in varying degrees. In some forms (cat, dog, calf, and man) it is almost impossible to determine the fate of pouch IV (excepting the parathyroid area) and the ultimobranchial body. The parathyroid component is usually easy to follow because of its early and distinctive differentiation.

The obscurity of the components of complex IV has lead some investigators to disregard and even deny their existence. Weller ('33) and Norris ('37) working with human material regard complex IV as a lateral thyroid and a parathyroid and believe that the "lateral thyroid" forms thyroid and has thyroid forming potencies. They completely ignore valuable comparative evidence and the earlier reports of a human thymus IV. Van Dyke ('41) has recently presented a very good account of the thymus IV in man. He does not however definitely assign the thymus IV to pouch IV exclusively. He even considers it plausable that ultimobranchial material would undergo thymic transformation.

In the opossum Kingsbury ('40) finds a separation of the ultimobranchial body and the pouch IV so that each can be followed. He finds that a large thymus IV clearly arises from pouch IV. The thymus IV in this form is even larger than thymus III. The conditions in the opossum are similar to that in the pig in that pouch IV and the ultimobranchial body separate so that each can be followed. As has been shown in the description of the pig pouch IV, when present, gives rise to thymus. In the pig therefore the thymus IV comes from pouch IV and only pouch IV. This is also true in the opossum. The author feels that this is true in all forms such as the cat, dog, calf, and man. This would seem to be the fate expected from the theoretical standpoint but the presence of thymic material in, or associated with, the thyroid in such forms as the cat, has apparently led to the now widely held belief that thymus material can form from pouch IV or the ultimobranchial body. In support of the conclusion that thymus IV comes from pouch IV only, it may be pointed out that the rat apparently forms no pouch IV but has a large ultimobranchial body (Rogers, '27, '29) and no thymus IV has been reported in this form. The author attempted to find a thymus IV but failed. If thymus forms from ultimobranchial material it would be present in or associated with the thyroid of the rat. This would also be true for the pig. In a study of the fate of the ultimobranchial body in the pig Badertscher ('18, '19) reported no thymus tissue in the thyroid of the pig. The simplest solution which is consistent with the known facts in the one stated above — that thymus IV forms from pouch IV and pouch IV only.

In the study of the origin of typical thymus structure the question always arises of what the first indication of thymic differentiation may be. In the pig the epithelial cells give the first sign of thymic transformation by becoming more basophilic in their staining properties and looser in their structure. Small spaces appear between the epithelial cells. The origin of the thymocytes was not determined although an attempt was made. Their origin from the epithelial cells must be regarded as a possibility in spite of much evidence to the contrary. Whether the epithelium forming thymus is in the wall of the pharynx or free in the connective tissue the process is the same. A very similar series of changes was found in a study of the origin of thymus IV in the dog (Godwin, '39). In the rat, Harlan ('40) found that the epithelium of thymus III formed a distinct reticulum before many lymphocytes were present. She believed that the reticulation was primary and not caused by the slight lymphocytic infiltration found in early stages. A discussion of the literature on this point may be found in her article.

An attempt was made earlier (Godwin, '40 a) to reach some conclusion regarding the factors which determine thymus formation. Little can be added to the earlier discussion. The epithelium of the pouch apparently carries thymus forming potencies which in all probability are determined during the early part of the branchial phase of pharyngeal development. It has been shown that in the pig the fourth pouch is variable in its development in the branchial phase and is frequently lacking on one or both sides. The pig is the only form known at present in which this peculiar mode of development exists. The stimulus for fourth pouch formation seems to be failing in this form. We also find that the development of thymus IV is sporadic and variable in the pig. This is known to be true of several forms even though their pouch IV does not show marked variations of development. The specimens studied are not of sufficient number to allow one to state the percentage of presence or absence of thymus on one or both sides but it can be said that thymus IV is present on at least one side in over half of the specimens and is therefore by no means rare. This compares favorably with the presence or absence of the pouch IV in the pig. It is obvious that a thymus will not form unless the material out of which it arises is formed. Again the case of the rat is pertinent; no pouch IV forms and no thymus IV is present. In some pigs this same situation is present. In others a pouch of varying degrees of development is present and a thymus of corresponding degree of development results from it. The original stimulus — strong or weak — probably results in a small poorly differentiated thymus or a large well developed thymus. The ultimate fate of thymus IV is not known in the pig or any other form.

The problem of parathyroid IV is closely related to that of pouch IV. In earlier stages a parathyroid IV could always be detected. In the latest stages no parathyroid IV seems to be present. Its absence was not expected but recent evidence in other forms is in line with this finding. Kingsbury ('40) reported that parathyroid IV in the opossum was small and variable despite the fact that thymus IV was larger than thymus III. McCrady ('41) was not able to locate parathyroid IV in the adult opossum. Schlotthauer and Higgins ('34) made a special study of the parathyroids in the pig and could not find a parathyroid IV. It seems probable that the parathyroid IV starts to develop but later degenerates in both the opossum and the pig.

CONCLUSIONS

1. The fourth pouch of the pig when present is a distinct development.
2. The fourth pouch is serially homologous with the third pouch.
3. The fourth pouch is variable in its appearance and may be absent on one or both sides.
4. The fourth pouch forms definitive thymus in late fetal stages.
5. The thymus IV is usually found in the subepithelial tissue near the dorsolateral and cephalic edge of the thyroid cartilage.
6. Frequently there is a partial thymic development of that part of the fourth pouch which remains in the epithelium in the dorsolateral wall of the laryngopharynx.
7. This intraepithelial thymic material indicates the exact point at which the fourth pouch becomes detached from the epithelium.

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PLATES

PLATE 1

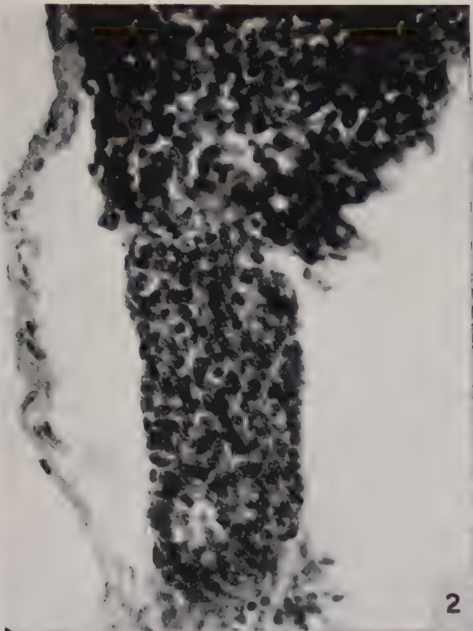
EXPLANATION OF FIGURES

2 A thick cord of pouch IV material is undergoing thymic change. The lower part of the picture shows epithelial cells with few or no thymocytes present. In the upper part of the picture thymocytes are plentiful and the cord of cells is greatly thickened as a result of their presence. × 367.

3 An epithelioid cluster of prethymus cells is shown at the edge of definitive thymus IV material. The epithelioid cells are like those in figure 2. There are many lymphocytes (thymocytes) in the connective tissue in the lower left quarter of the figure. × 367.

4 A small definitive thymus IV lies in a break in the pharyngeal musculature. Cortex and medulla are present. An arrow indicates a small patch of prethymus cells near the point on the pharyngeal wall where pouch IV become detached. A piece of the pharyngobranchial duct is present on the right edge of the cell cluster. The cephalic edge of the thyroid cartilage shows at the lower right. Frontal section. × 83.

5 A small partially developed, and therefore at present atypical, thymus is shown lying adjacent to a prethymus area in the epithelium of the pharyngeal wall. The surface region of the intra-epithelial thymic region has many thymocytes. Figure 9 shows a higher magnification of this region. × 166.



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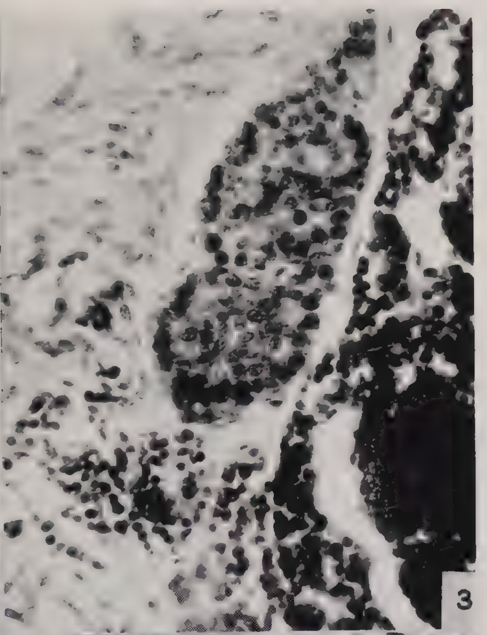

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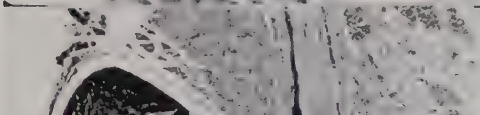

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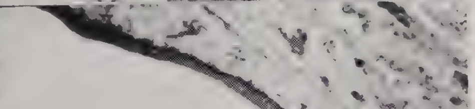

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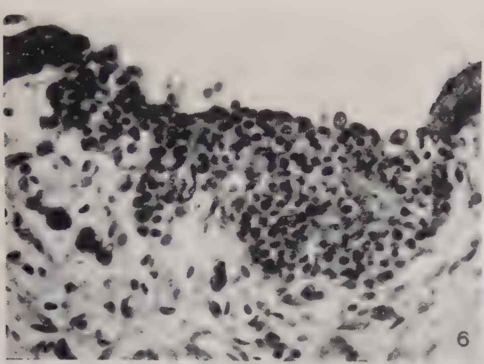
PLATE 2

EXPLANATION OF FIGURES

6, 7, 8, 9 These figures show intraepithelial prethymic areas. All are small and are abruptly set off from the stratified squamous epithelium of the laryngopharynx which surrounds them. In figure 6 very few thymocytes are yet present but the epithelial cells have become loosely arranged and many have become more basic in their staining reaction. In figures 7 and 8 many thymocytes are present particularly near the surface. Figure 9 is a high power of a part of figure 5. In this figure the surface region of the intraepithelial thymus is packed with thymocytes. Several small darkly staining cells are present in the connective tissue in the lower center. A remnant of the pharyngobranchial duct is present at the lower right. × 293.

10 This frontal section through the pig larynx shows a definitive thymus IV on both sides located under the epithelium of the laryngopharynx near the cephalic edge of the thyroid cartilage. Lobulation and cortex-medulla are easily visible in the thymus. A small piece of the ultimobranchial body is present caudad to the point of articulation of the thyroid cartilage with the cricoid cartilage. × 4.2.

11 This figure is a high power of a part of figure 10. It shows a well developed Hassall's corpuscle. × 293.



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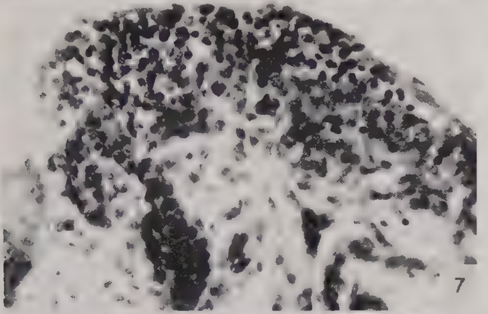

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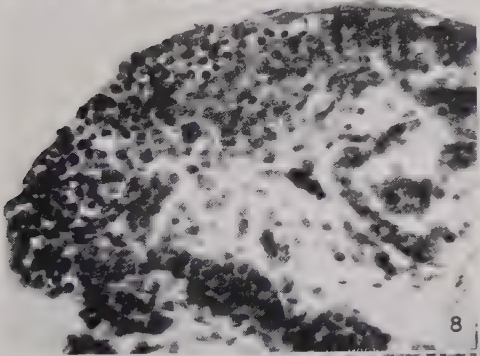

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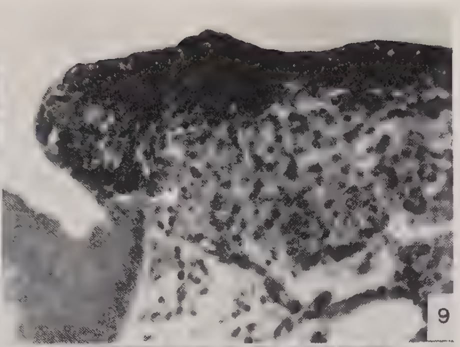

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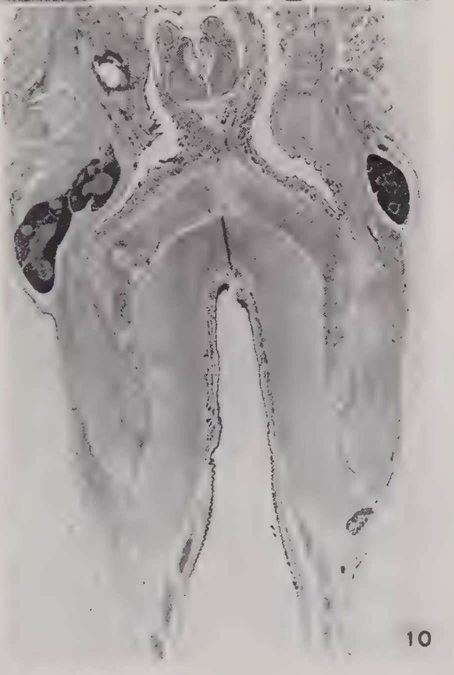

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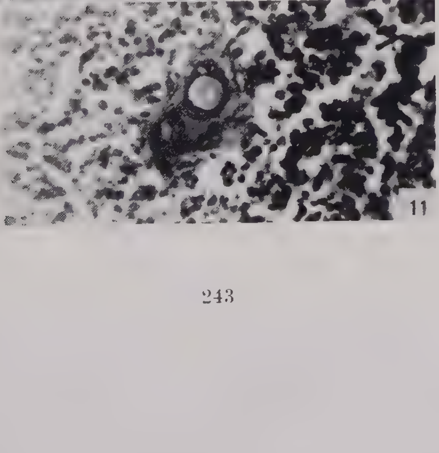

11

REACTION OF ORBITAL TISSUES IN EXPERIMENTAL EXOPHTHALMOS FOLLOWING REMOVAL OF HARDER'S GLAND[1]

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It has been shown that the injection of anterior pituitary extracts into thyroidectomized guinea pigs results in a marked and sustained exophthalmos (Marine and Rosen, '34; Smelser, '36; Paulson, '37; Aird, '41) which is strikingly similar to that found in man, particularly in those cases which persist or progress after thyroidectomy. The orbital fat and muscles of such animals hypertrophy and are edematous as in the clinical cases (Smelser, '37). The exophthalmos and the orbital changes are not produced by neural stimuli passing through the cervical sympathetic ganglia or by contraction of the smooth orbital muscle of Müller (Smelser, '39 a) — factors which are minimal or not involved in the human. In addition to the hypertrophy of the orbital fat and muscles there is an increase in weight (Ca 40%) of a large orbital gland (Harder's) under the influence of injected anterior pituitary extracts. Because of its size and retrobulbar position the hypertrophy of Harder's gland undoubtedly contributes to the exophthalmos produced by anterior pituitary extract injection. Therefore, it seemed desirable to determine whether or not this type of exophthalmos was dependent upon the presence of this orbital gland or whether hypertrophy of fat and muscles alone could be responsible for the proptosis.

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[1] Aided by a grant from the Francis I. and Elizabeth C. Proctor Fund.

EXPERIMENTAL

The harderian gland was removed from the right orbit of a number of 200–300 gm. guinea pigs which had been thyroidectomized 4–6 days previously. The method of removing Harder's gland varied somewhat between the earlier and later experiments. At first the entire gland was removed through an incision in the skin and Müller's orbital muscle, approaching the orbit from below. After reflecting the lacrimal gland an excellent exposure of the lower part of the orbit is obtained. The relationships of the various structures in this region relevant to this operation have been described (Smelser, '39 a). Although the entire gland can be withdrawn from the orbit when this exposure is used, fragments were frequently left at the point where the secretion products are discharged in the nasal part of the conjunctival sac. Therefore in the later operations a small second incision was made at the same time through the conjunctiva at this point, and that part of the gland removed through the second opening. The gland was removed through an approach to the inferior position of the orbit because its major portion lies below the muscle cone and quite temporally and also because it was desired to partly replace the gland with fat taken either from the same animal or a closely related donor. It appeared possible to place such grafts into the orbit with least handling of the implanted tissue through the fairly open inferior approach, thus making it possible to have some assurance of the final location of the grafted tissue. Fat was implanted in the orbit following ablation of the gland to counteract the marked enophthalmos which results from this operation. The grafts were weighed (100 mg. average) before implantation but completeness of their vascularization and subsequent maintenance probably varied. In nine cases the fat used to replace Harder's gland was obtained from the ovario-uterine mesentery of the operated animal. In fourteen additional cases homeo-transplants of orbital fat from parental donors were made.

Six to 8 weeks after the orbital operation some animals of each group were given daily subcutaneous injections of an anterior pituitary extract. The material, prepared as described earlier (Smelser, '38) was made from acetone dried bovine anterior pituitary glands by aqueous alkaline extraction followed by isoelectric precipitation. The active material, found in the isoelectric soluble portion was precipitated with flavianic acid and injected as a flavianate. The daily dosage was 20 mg., equivalent to 400 mg. of the original acetone dried powder. This extract contained a large amount of thyrotropic hormone, and also adrenotropic and gonadotropic hormones, the latter causing mainly luteinization.

In addition to the above experiments a number of newborn guinea pigs were subjected to ablation of Harder's gland from the right orbit without subsequent implantation of fat. All of these operations were done on animals less than 24 hours old at which time the gland is small and can be easily withdrawn from the orbit through a small incision in the nasal portion of the conjunctival sac without bleeding and with very little trauma. Although removal of Harder's gland at birth causes some enophthalmos it is not as marked as that which followed the operation in older animals. This is probably due to the small size of the gland at birth, its removal therefore involving but little damage to other structures. Following its ablation the orbital fat hypertrophies, partially compensating for loss of the gland. When the animals had reached a weight of 450–500 gm. they were thyroidectomized, part of each litter was reserved as controls and the others were injected daily with anterior pituitary extract as were the first groups.

In these three series it was possible to study the effect of anterior pituitary extract on the production of exophthalmos and on the hypertrophy and pathological modification of the orbital fat and muscles in the absence of Harder's gland. In each case the essential comparisons are made between the operated and intact orbits of the same animal.

At the conclusion of the experiment the width of the palpebral fissure was measured and the animals autopsied. The orbital tissues were weighed and fixed for histological examination. Measurements of the palpebral fissure width are easy to obtain with vernier calipers and the values can be repeated within a narrow range if they are determined under fairly constant conditions of light and quiet. The degree of exophthalmos was estimated, in addition to inspection, by measuring the distance from the supraorbital notch of the skull to the nearest point on the limbus. Since this determination was made after skinning and removing the head from the body, congestion of the orbital veins or retraction of the lids are removed as factors in the cause or appearance of exophthalmos. If these measurements are made when the eye is not deflected the values are an objective criterion of the degree of protrusion of the globe from the bony orbit.

A definite exophthalmos was produced and maintained to the end of the experiment in the unoperated eye by the injection of the anterior pituitary extract in all three groups. The effects produced in these orbits confirmed fully earlier observations. Thus the palpebral fissure which in the normal guinea pig of this size is about 7.8 mm. was increased to 8.7 mm. ($P = < .01$)[2] in the exophthalmic animals, table 1 A. It is emphasized that this comparison was made between animals of similar body and eyeball size and in more than half the cases between litter mates. The increase in palpebral fissure width is the most obvious feature in exophthalmos, since it exposes a band of sclera and thus creates a staring expression.

All of the cases reported were definitely exophthalmic as judged by both appearance and the skull limbus measurements. Reference to the table shows that the proptosis produced was marked, averaging 4.8 mm. compared with 3.3 mm. of the uninjected controls. The difference, 1.5 mm. is statistically significant ($P = < .01$).

Both the orbital fat and muscles were hypertrophied, the fat being approximately doubled in weight and the muscles increasing in weight by 20%. Both of these increases are statistically valid ($P = < 0.1$) and of the same order of magnitude as reported in earlier experiments. The lacrimal gland which, in the guinea pig is lateral to and below the globe, and is essentially outside of the orbit, was unaffected by the injections, having a mean weight of 218 and 212 mg. in the control and experimental animals respectively. Harder's gland, however, increased 42% in weight on the unoperated side and because of its large size it was a major factor in the production of exophthalmos in that eye. The structural

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[2] ''P'' represents the probability that the differences between two means is due to random sampling and is calculated here according to methods outlined by Fisher in ''Statistical Methods for Sesearch Workers.'' Oliver and Boyd, Edinburgh 1934. A probability of 0.05 (5 chances in 100 that random sampling is responsible for the difference) or less is frequently accepted as indicative that the difference is statistically significant.

TABLE 1

The effect of anterior pituitary gland extracts on the orbital contents following ablation of Harder's gland.

	NO. CASES	BODY	GLOBE	LACRIMAL GLAND	PALPEBRAL FISSURE	SKULL-LIMBUS	ORBITAL FAT	EXTRA-OCULAR MUSCLES	HARDER'S GLAND	TOTAL RETROBULAR TISSUE
		gm.	*mg.*	*mg.*	*mm.*	*mm.*	*mg.*	*mg.*	*mg.*	*mg.*
A. Intact orbits (left orbits)										
Control	25	524	459	218	7.8	3.3	46	83	374	502
Experimental	23	509	457	212	8.7	4.8	88	99	533	720
$\epsilon_{\text{Diff.}}$					.15	.16	6	4	27	31
B. Ovario-uterine fat implant (right orbits)										
Control	4	445	444	219	6.1	2.5	303	75	...	378
Experimental	5	426	485	152	7.4	3.7	459	88	...	547
$\epsilon_{\text{Diff.}}$					.6	.4	85	7		67
C. Orbital fat implant (right orbits)										
Control	9	465	449	190	6.7	2.9	159	78	...	237
Experimental	5	378	447	174	7.5	3.9	243	88	...	331
$\epsilon_{\text{Diff.}}$					.5	.4	42	6		34
D. No implant (right orbits)										
Control	12	595	488	264	6.6	2.6	131	85	...	217
Experimental	13	592	483	269	7.3	3.4	201	99	...	300
$\epsilon_{\text{Diff.}}$					.17	.20	17	5		20

$$\epsilon_{\text{Diff.}} = \sqrt{\left(\frac{\Sigma d^2}{n(n-1)}\right)^{\text{Cont.}} + \left(\frac{\Sigma d^2}{n(n-1)}\right)^{\text{Exp.}}}$$

changes in the harderian gland which result from these injections have been reported (Smelser, '41).

A definite proptosis likewise was produced on the operated side from which Harder's gland had been completely removed. It must be held in mind that an enophthalmos existed in all of these eyes before the injections began and that the exophthalmos was judged relative to the operated controls although a proptosis was produced which was actually greater than that of the unoperated eye.

The exophthalmos produced was independent of the presence of a fat graft of any type. The first group, those in which Harder's gland had been partially replaced by ovario-uterine fat were markedly exophthalmic as shown by the mean skull-limbus measurement of 3.7 mm. as compared with 2.5 mm. of the uninjected controls bearing similar grafts. There was an increase in width of the palpebral fissure which was relatively more marked than on the intact side. The orbital fat, including the transplant, was larger in the exophthalmic animals increasing from 303 mg. to 459 mg. or 51% (table 1 B). Its percentage of hypertrophy is less than that of the fat in the intact orbits but the large original size of the mass of fat probably limits its increase. The weight of the extraocular muscles also increased nearly although not quite as much as those in the intact orbits and although these changes are comparable to those obtained in the unoperated orbits, the number of cases is too small to provide statistically valid differences. The lacrimal glands in these orbits, as in the unoperated ones, were unaffected by the injections; the slight difference shown for this group in the table is apparently accidental and did not occur in the other series.

Those animals in which Harder's gland had been replaced by a transplant of orbital fat behaved essentially as did the animals just described. An exophthalmos was produced on the intact left side of all the injected animals. In one case, however, it was very slight with commensurate changes in the orbital tissues of both unoperated and operated orbits. The width of the palpebral fissures of this group was increased on both sides in the injected animals. That of the operated eyes increased 0.8 mm. over the uninjected control width (table 1 C), an increase which compares favorably with that obtained (0.9 mm.) in the intact side. An excellent proptosis was obtained, in the absence of Harder's gland. The skull to limbus value of the injected animals (3.9 mm.) not only exceeded that of the operated, but was greater than in the unoperated controls (3.3 mm.). The significance of the difference ($P = ca. .02$) is strengthened by the fact that the eyeballs of the injected and control animals were of the same size, and the weight of the lacrimal glands did not differ appreciably. The fat in the orbit which fills almost all of the retrobulbar space, and includes both original and transplanted fat, increased in weight 52% (table 1 C). There was thus the same degree of hypertrophy as in the injected animals with ovario-uterine fat transplants. The extra-ocular muscles likewise hypertrophied, actually more than in the intact orbits of the same animals. The increase in the weight of all the retrobulbar tissues amounted to 40% which is essentially the same as in the preceding series (42%) as well as in the series with Harder's gland in situ (42%).

In the last, and most extensive, series Harder's gland had been removed at birth and fat was not transplanted into the orbit. These animals reacted very much as did those in which a transplant of fat had been made to the orbit. Since the control and experimental animals of this group were litter mates, closer correlation in body size and organ weights might be expected and consequently greater significance attached to the effects of the injected extracts. The pituitary preparation as in the other experiments, had no effect on the body weight or the size of the eyeball or the lacrimal glands. All of the changes associated with this type of exophthalmos, however, were produced in the complete absence of Harder's gland and transplanted fat. There was an increase in width of the palpebral fissure of 0.7 mm. ($P = < .01$) and in the protrusion of the globe of 0.8 mm. ($P = < .01$) (table 1 D).

There was also the characteristic hypertrophy of the orbital fat, in this series amounting to 66% over the controls ($P = < .01$). It should be emphasized that this hypertrophy was superimposed upon an increase in the fat which resulted from ablation of Harder's gland and which, of course, occurred in the orbits of injected and uninjected animals alike. The 66% hypertrophy of the orbital fat in this series, which resulted from the injected pituitary extract was a more marked response than in the other two groups.

A possible explanation of this greater reaction may be that this tissue had been subject to less trauma and consequent growth of connective tissue and interruption of the normal blood supply than had been the case in the transplant series. There was also a definite hypertrophy of the extraocular muscles (16%, $P = < .01$) comparable to that obtained in the other groups but slightly less than in the intact orbits. The total retrobulbar tissue was thus increased 39% ($P = < .01$), a value again comparable to that obtained in orbits possessing a Harder's gland. All of these changes in fissure width, proptosis and orbital tissue hypertrophy were sufficiently great and consistent to be statistically significant and were, relative to their controls, as marked as the effects obtained in the unoperated orbits.

The orbital tissues of the operated and the unoperated orbits were studied in all three series. In each case the six extraocular muscles and levator of both orbits were doubly embedded in celloidin and paraffin, sectioned at 3 μ and stained by Masson's trichrome method. The orbital fats were studied in similarly stained 6 μ paraffin sections. A few additional hematoxylin and eosin preparations were also made.

The histological modification of the orbital muscles in experimental exophthalmos have been reported (Smelser, '37, '39 a). These changes consist mostly in an increase in interstitial fluid or edema. Since the extraocular muscles of all three groups and of the operated and intact orbits reacted identically they are considered together. In the majority of thyroidectomized uninjected guinea pigs a small amount of stainable material can be found between the fibers of some of the muscles. Usually two or three of the seven extraocular muscles may show this condition. Injection of anterior pituitary extract apparently increased both the amount of this interstitial fluid and its distribution so that instead of a few, nearly all of the extraocular muscles were affected, producing areas of marked edema. The edema fluid appeared as a stainable coagulum, indicating that it contained considerable protein. Usually the tendinous end of the muscle was more edematous than the other parts, the infiltrate being associated with the connective tissue bands. However, it was by no means limited to that area and occurred throughout the muscle. Leucocytes were occasionally found associated with the edema, not only in the operated orbits, with transplants, but also on the unoperated side. In spite of the edema of the muscle as a whole, the structure of the individual fibers was surprisingly normal. With rare exceptions the fibers had excellent and easily demonstrable striations, myofibrils and sarcosomes, the nuclei also rarely diverging from normal. In a few instances muscles were found which lacked the normal architecture, but the number of affected fibers in them was very small. These affected fibers were swollen, irregular in outline, and their cytoplasm appeared to be quite structureless, staining unevenly. The fibers could be followed for long distances in the sections without finding traces of striations or fibrils, and with only an occasional shriveled nucleus. The significance of the occurrence of such fibers is obscure. The possibility that they represent a technical artefact does not seem likely but cannot be wholly disregarded.

All of the morphological changes described were present in the orbital muscles of the injected animals whether or not Harder's gland had been removed or fat implanted. It thus is evident that these effects were not the result of trauma associated with removal of the gland nor were they caused by the implantation of the fat grafts. This point is of particular importance in interpreting the leucocytic infiltration and the occurrence of atrophic muscle fibers. A consideration of the changes produced in the orbital fat requires reference to the normal condition.

In the guinea pig this tissue differs somewhat in character from the fat depots found in other regions of the body. There is a good deal more loose connective tissue, which tends to divide the fat cells into lobules, than, for example, in the adipose tissue surrounding the kidney. Following Bouin fixation a small amount of stainable intercellular fluid can be found in the interstices of this loose tissue of uninjected, thyroidectomized animals. The normal character of this tissue was unaltered by removal of Harder's gland at birth. Although ablation of this gland led to an increase of about three times the amount of orbital fat this increase was a normal hypertrophy and resulted apparently as an attempt to replace the harderian gland as a cushion for the globe. Excessive amounts of connective tissue were not found in the orbits of the animals of this series.

There was, however, slightly more connective tissue found in the orbits in which ovario-uterine fat implants were made than in the other, doubtless a reaction to incorporation of the graft. The implants of this type of fat became well vascularized and healthy without exception. They seemed to preserve their identity, however, as their firmer consistency differentiated the graft somewhat from the native orbital fat. Histologically the fat from ovario-uterine mesentery is a more solid adipose tissue than that of the orbit, the lobules of fat cells are larger and there is less loose connective tissue. This structural character of the tissue was more or less retained when moved to the orbit. Contrary to the success had in making these transplants, several cases of the orbital fat series were found in which the grafts had been completely resorbed. In these cases, which are not included in the reported data, there was a marked enophthalmos and a great deal of connective tissue in the operated orbit. In those cases in which the orbital fat graft did become incorporated a more or less severe reaction appeared to have taken place, possibly due to the fact that these grafts were not auto transplants.

There was, in all, some proliferation of connective tissue and associated with this reaction, considerable edema. For this reason it is difficult, in this particular group, to evaluate the histological changes in these grafts in response to the injected pituitary hormone and the consequent exophthalmos.

Injection of pituitary extracts provoked typical and equal reactions in the fat of the orbits in which no graft replaced the extirpated Harder's gland as well as in the fat of the intact orbits. For this reason and because in both cases the tissue is all original orbital fat they are described together.

The orbital fat of the exophthalmic animals showed an extreme edematous infiltration which apparently accounts for almost all of the increase in weight of that tissue. The edema penetrates between the loose connective tissue fibers separating them so that large areas can be seen in sections which are almost pure edema fluid, containing a few fibroblasts and leucocytes. The intercellular spaces between the fat cells also are penetrated by the fluid forcing them apart in such a manner that many appear to be almost isolated from each other. This infiltration is very even, affecting all parts of the orbital fat depot but is most extreme in the connective tissue and less so among the solid masses of fat cells. Insofar as can be judged in sections the edema of the fat is of the same nature as that found in the muscle. With Bouin fixation it appears as a smooth stainable coagulum, indicating a large protein content. Photomicrographs illustrating the appearance of the edematous infiltration in orbital fat and muscles have been published (Smelser, '37, '39 a, '43).

There is, in addition to the edema of the fat, some increase in connective tissue in the exophthalmic animals. All of the above changes were found equally developed in the fat of intact orbits and in those lacking a Harder's gland but in which no graft had been made.

Sections of the orbital fat from those animals in which it was made up in part by a graft of ovario-uterine fat showed a somewhat different reaction. As pointed out above there was some increase in connective tissue in the grafts in the control animals, and a similar condition was noted in the injected group. Contrary to the finding in the animals which bore no graft the edematous change in the fat did not affect all parts of the orbit equally. In each case the orbital tissues consisted of two types of fat tissue, one in which there was rather slight edematous infiltration and not much of the loose connective tissue so characteristic of orbital fat. The rest of the section was markedly edematous, showing all the changes found in the fat from intact orbits of animals treated with anterior pituitary extract. This suggests that these parts represent grafted and original fat, tissues with inherently different capacities to react to the pituitary extract. This uneveness in response did not occur, however, in the group of animals which bore grafts of orbital tissue. In these the edematous and cellular infiltrations plus proliferation of connective tissue were found to an extreme degree and equally developed in all parts of the orbit. Excepting, perhaps, for the greater connective tissue proliferation, all the fat which filled the orbit reacted as did the fat in the unoperated orbit of the same animals.

DISCUSSION

The exophthalmos which is produced in thyroidectomized guinea pigs by the injection of anterior pituitary extracts is accompanied and apparently caused by an hypertrophy of the retrobulbar tissues. Of the three reacting structures, two, the fat and the muscles, are tissues common to all parts of the body, but are affected mostly in this particular location while the other, Harder's gland, is specifically an orbital structure. There can be no doubt, as was early recognized (Smelser, '37, '39 a) that the hypertrophy of this gland is an important contributing factor to the exophthalmos in the guinea pig. This fact introduces a somewhat anomalous note into the general picture of thyroid-pituitary physiology in relation to exophthalmos. Not only is the function of Harder's gland not clearly known but its relationship to the pituitary can only be conjectured. It is possible that the apparent association of the harderian gland with exophthalmos is purely fortuitous, certainly it does not seem to occupy an important position in the exophthalmic goiter syndrome.

That Harder's gland is not a necessary factor in the production of exophthalmos in the guinea pig is shown by the experiments reported here. It is noteworthy that all of the signs and changes associated with exophthalmos, in the experimental animals were produced after removal of this gland with or without replacing it with fat, thus indicating that none of them were dependent upon Harder's gland. This does not prove that all of the characteristic changes in the fat and muscle are basic to the exophthalmos for some of them may result from the proptosis. For example: the hypertrophy of the extraocular muscles has been shown to be in part, a response to the forward movement of the globe (Smelser, '42, '43) and certainly the width of the palpebral fissure could similarly be increased by the same action.

The exophthalmos itself seems to be dependent upon hypertrophy of the retrobulbar tissues especially the fat, and this, in turn, upon the accumulation of water in this tissue. This sequence of events, as the present data show, is clearly independent of the presence of Harder's gland.

The involvement of the extraocular muscles has been emphasized, particularly in relation to clinical exophthalmos (Moore, '23; Burch, '29; Naffziger, '33; Thomas and Woods, '36). Although there is no doubt that hypertrophy and pathological changes occur in these muscles in experimental exophthalmos, it appears necessary to conclude that the edema is comparable to, although less than that of the fat, and it seems reasonable to suggest that both are brought about by a similar mechanism. The pathology of the individual muscle fibers, stressed by Aird ('41) is difficult to evaluate. Certainly the extent of the atrophy found is very limited and its infrequent occurrence requires that it be interpreted very cautiously. Atrophy of the muscle fibers would contribute to the degree of exophthalmos if it were sufficient to weaken the retractor action of the recti muscles.

Study of the grafts of the two types of fat used to replace Harder's gland introduces some interesting possibilities. The hypertrophy of the orbital fat homeo-transplants show that the influence of the pituitary extract is effective on tissue which has had its nerve and blood supply disturbed by grafting. The edema produced in these grafts was extreme and evenly distributed. Examination of the ovario-uterine fat transplants, however, showed that this tissue was not as readily affected as is the orbital fat. This is in agreement with previously reported data which showed that in exophthalmic animals adipose tissues from other parts of the body were less edematous than the orbital fat (Smelser, '39 b). This greater reaction of the orbital fat to the injected pituitary material would account for the rather restricted region in which the major effect is found.

I wish to express my indebtedness to Miss Victoria Ozanics for her excellent assistance throughout this study.

SUMMARY

1. The presence of Harder's gland is not necessary to the exophthalmos, caused by the injection of anterior pituitary extracts, or to the concomitant increase in palpebral fissure width or to the hypertrophy of the orbital fat and muscles.

2. Pathological modification of these tissues, mainly edematous infiltration, occurs as readily and as extremely in the absence of Harder's gland as in the intact orbit.

3. Grafts of orbital fat react to the injected hormones as uniformly as does native orbital fat.

4. Intraorbital grafts of fat from the ovario-uterine mesentery do not become as markedly edematous as orbital fat.

5. Ablation of Harder's gland has no apparent deleterious effect on the eye.

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GROWTH OF AVIAN OVUM

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THREE FIGURES

The growth of the avian ovum is a continuous process. Its rate of growth is very slow until it reaches the maturation stage. In this final period the actual growth, or deposition of deutoplasm, takes place with great rapidity, the ova reaching their full size within 5 or 6 days.

The development of the chicken (Gallus domesticus) ovum has been studied by several workers (His, 1868; D'Hollander, '04; Sonnenbrodt, '08; Riddle, '16; Stieve, '18; Romanoff, '31; Warren and Conrad, '39). Chomkovic ('28) following the growth of the ovum in ducks (Anas domesticus), found it similar to that in chickens. The growth of the ovum in other birds as yet has never been investigated, although the knowledge about the quantitative daily depositions in several species would be of value in determining the growth pattern of the avian ovum in general. The present discussion therefore is based on additional data of two species, the ring-necked pheasant (Phasianus torquatus), and the bobwhite quail (Colinus virginianus).

Growth of ova in various species

The avian ovary during the optimum egg-laying period possesses a series of ova in ascending stages of development. When eggs are laid daily these ova may be found in corresponding growth phases. The weights of individual ova from the ovaries of pheasant, quail, chicken and duck are shown plotted against their descending order of formation (fig. 1). Since the weight of the egg and of its yolk is proportional to the weight of the bird the curves differ according to the size of the bird, being largest in the duck and smallest in the quail. The L on the abcissa represents the ovum or yolk of a new-laid egg. If an index of growth rate is used

$$\frac{\text{weight of ovum 1} - \text{weight of ovum 2}}{\text{weight of ovum L (yolk)}} \times 100$$

so that a comparison of species may be made, then it is found that the growth-rate of the ovum is practically identical for all the species of birds studied (fig. 2).

These growth-rate curves of the ovum of nudifugous (or chick-producing) birds may not be applicable to those of

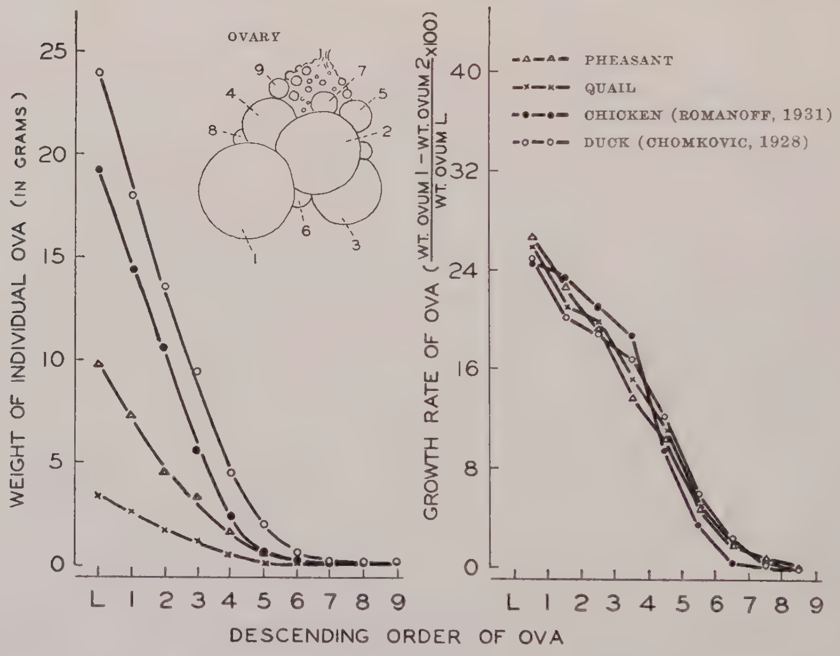

Figure 1 Figure 2

Fig. 1 Weights of individual ova in the functioning ovaries of pheasant, quail, chicken and duck plotted in descending order. The L indicates the ovum (yolk) in the new-laid egg.

Fig. 2 Relative growth-rate of ova of pheasant, quail, chicken and duck.

nidicolous (or squab-producing) birds, like the pigeon (Bartelmez, '12) or the starling (Bissonnette and Zujko, '36). In birds of the latter group laying small clutches of eggs, the follicles are arranged in sets, and may not be in all stages of continuous growth.

Shape of ovum

It was a common traditional view, as Bartelmez ('18) stated, that the shape of the yolk is a sphere. Now it is generally accepted that it is far from being spherical. Not only is its longest axis perpendicular to the polar axis but it is usually somewhat flattened at the animal pole. Thus the polar axis is its shortest axis. Moreover von Baer's (1828) observations show that the yolk is ovoid rather than oval in shape — the more pointed end corresponding to the pointed end of the shell. The data for the dimensions of chicken ova in the latest stages of development and in the laid egg are given in table 1. The shapes of the ova (enclosed in follicles) were measured after coagulating them in boiling water.

TABLE 1

Dimensions and dry matter content of growing avian ova.

DESCENDING ORDER OF OVA	DIMENSIONS OF OVA (CHICKEN)			DRY MATTER CONTENT		
	Along long axis of egg	Animal to vegetative pole (polar axis)	Perpendicular to polar axis	Chicken (Romanoff, 1931)	Pheasant	Quail
	mm.	*mm.*	*mm.*	*per cent*	*per cent*	*per cent*
In laid egg	34.0	31.0	32.0	52.36	52.61	53.87
In ovary – 1	34.0	28.0	29.0	54.54	55.24	56.63
2	31.0	26.0	26.5	53.20	54.52	53.54
3	24.0	20.5	21.5	51.05	53.42	51.44
4	19.0	15.0	16.0	48.15	51.24	42.74
5				38.30	37.96	
6				18.57	20.77	
7				17.60	17.43	
8				15.18	16.43	
9				14.45	15.45	

Dry matter content

As has been previously shown for chicken ova (Romanoff, '31), there is a rapid increase in percentage of dry matter during the maturation phase of both pheasant and quail ova (table 1). Following the maximum percentage in the largest ova, the values drop to those usually found in new-laid eggs. This agrees essentially with data presented by Spohn and Riddle ('16) in which values were obtained for isolated stages in development of fowl ova.

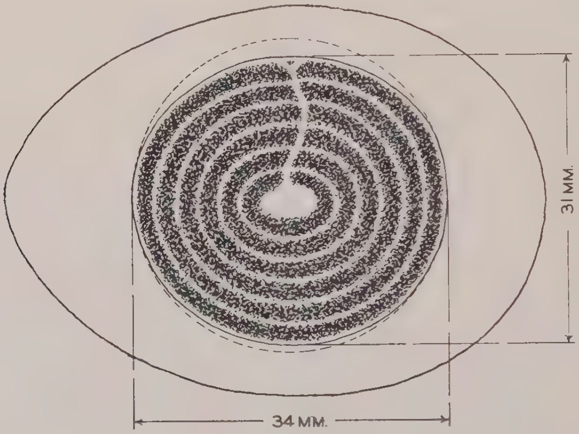

Fig. 3 Dimensions and stratification of the ovum (or yolk) of a new-laid chicken egg.

Stratification of ovum

Studies of avian yolk by means of deposition of Sudan III through feeding (Gage and Gage, '08), or intravenous injection (Warren and Conrad, '39) reveal that it has a stratified structure composed of alternating light and dark concentric layers of the white and yellow yolk (fig. 3). They are arranged nearly symmetrically except for the interruption at the germinal disc and surrounding the latebra. The thinner white and thicker yellow yolk layers (in the chicken egg about 0.4 mm. and 2.0 mm. thick, respectively) are deposited during each 24 hours, the former being produced from midnight to early morning, and the latter during the rest of the day. The deposition occurs according to a daily nutritional rhythm, the thinner white yolk being formed when the food supply is reduced to a minimum, and the thicker yellow yolk being laid down during the period of maximum food supply. The efficient metabolism of the hen permits a rapid alteration in deposition upon resumption of feeding. On the basis of the presented data (fig. 1) it is possible to visualize that the concentric rings of yellow and white yolk represent the actual successive daily growth of the ovum, as found in the ovary.

TABLE 2

Assimilation of ovarian follicles following discharge of ova.

DESCENDING ORDER OF FOLLICLES IN OVARY	WEIGHT OF FOLLICLES	
	Pheasant	Quail
	grams	*grams*
1	0.170	0.053
2	0.078	0.014
3	0.047	
4	0.016	
5	0.007	

Absorption of ovarian follicles

The follicle is a sac which encloses a single ovum and plays an important part in the nutrition of the egg until the ovum attains full size. After the discharge of the ovum the follicle collapses and begins to degenerate. The absorption is quite rapid, as shown from the data on pheasants and quail (table 2). According to Pearl and Schoppe ('21), however, there was found in White Leghorns (Gallus domesticus) on an average as many as eighty-seven discharged follicles of various sizes. Nevertheless, due to the eventual disappearance of these follicles, it would be impossible from the number of free follicles present in the ovary to determine the total number of ova discharged by an individual bird as has been attempted in the past.

SUMMARY

The rate of growth of ova in an active ovary is essentially identical in pheasant, quail, chicken and duck.

The shape of ovum is not spherical but slightly elongated and pointed towards the sharp end of the egg, and with a depression at the animal pole.

The concentric stratifications of the ovum closely correspond to successive sizes of ova found in the functioning ovary.

The ovarian follicles after the discharge of the ova are completely reabsorbed.

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A NOTE ON THE FORMATION OF THE PLANTAR ARTERIAL ARCH OF THE HUMAN FOOT

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TWO FIGURES

INTRODUCTION

There has been a tendency to accept without question many anatomical descriptions which have found their way into standard textbooks of anatomy. Such descriptions, even though they may be faulty, are often passed on from textbook to textbook.

Early textbooks of anatomy (Bell, 1812, and Cruveilhier, 1844) as well as modern texts (Gray, Cunningham, Piersol, and Morris) have uniformly described the plantar arterial arch as being formed by the lateral plantar artery. Observations made in the course of routine laboratory and special dissections, however, have indicated to the author that the mode of formation of the plantar arch as described in many textbooks may not conform to the conditions existing in most of the cases, and that the deep plantar branch of the dorsalis pedis artery plays the major part in the formation of this arch.

MATERIAL AND METHODS

The observations described in the following study were made on 361 feet dissected by the medical students of Tulane University during the years 1926–1927, by the students in Wake Forest College School of Medicine, 1928–1938, and also include 150 feet dissected and studied by the author at Jefferson Medical College in April, 1939. In numerous instances dissection followed the use of special injections into the arteries. The size of the lateral plantar artery at the base of the fifth metatarsal bone was noted, as was also the size of the deep plantar branch of the dorsalis pedis on reaching the sole of the foot at the proximal end of the first interosseous space. The arterial arch between these two points was closely observed, note being made as to whether or not there was a diminution in size from medial to lateral limits of the arch or vice versa, and also the manner of origin of distributing branches from the arch. These observations were recorded on mimeograph drawings of the foot.

The following figures are pen drawings of the dissected foot, plantar surface.

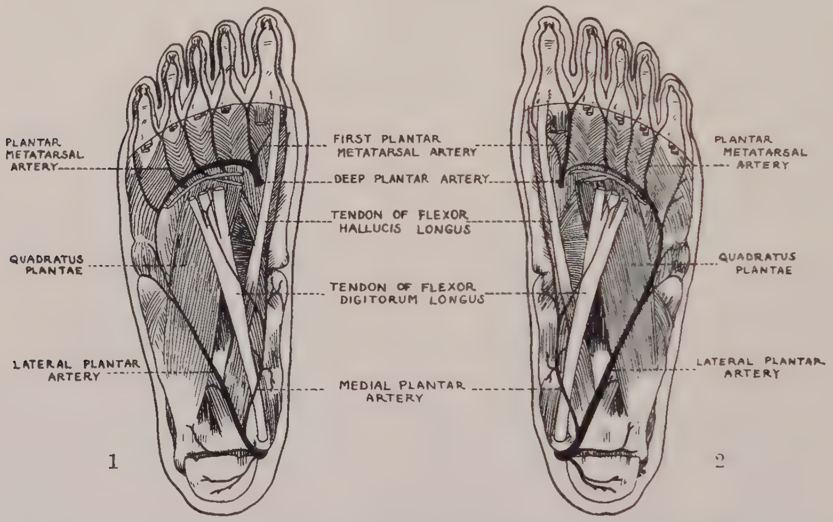
PLANTAR METATARSAL ARTERY
FIRST PLANTAR METATARSAL ARTERY
PLANTAR METATARSAL ARTERY
DEEP PLANTAR ARTERY
TENDON OF FLEXOR HALLUCIS LONGUS
QUADRATUS PLANTAE
QUADRATUS PLANTAE
TENDON OF FLEXOR DIGITORUM LONGUS
LATERAL PLANTAR ARTERY
LATERAL PLANTAR ARTERY
MEDIAL PLANTAR ARTERY
1
2

Fig. 1 The deep plantar branch of the dorsalis pedis artery is represented here crossing from the medial to the lateral side of the foot and playing the major part in the formation of the plantar arterial arch as was found in 80.887% of the cases.

Fig. 2 The course of the lateral plantar artery and its anastomosis with the deep plantar branch of the dorsalis pedis artery at the level of the first interosseous space as it was found to occur in only 15.235% of the cases.

OBSERVATIONS

First series: 211 feet (sex and race not recorded)

In 86.7% of the feet examined in this series, the point of smallest diameter in the arteries forming the arch was found to be located near the base of the fifth metatarsal bone (fig. 1).

The medial portion of the arch was, therefore, larger than the lateral, and the deep plantar artery appeared to diminish in size as it crossed the foot to the base of the fifth metatarsal bone. The plantar metatarsal arteries, in general, were observed to arise from the deep plantar portion of the arch and pass forward to the toes. The departure of these arteries from the arch was marked by a slight angle corresponding to the direction of blood current, i.e., lateralward.

In 10.4% of the feet the arch was formed primarily by the lateral plantar artery (fig. 2). Likewise, the plantar metatarsal arteries were found to be given off from the lateral plantar portion of the arch in line with the blood current, i.e., medialward.

The point of smallest diameter in the remaining 2.8% of the cases was doubtful, and therefore they are not considered in this paper.

Second series: 150 feet (sex and race recorded)

This series includes 89 white and 61 negro feet; 135 male, 15 female; 77 right, and 73 left. The dorsalis pedis artery played the major part in the formation of the plantar arch in somewhat varying percentages, as shown in the following tables:

Sex (150)

	MALE		FEMALE	
		%		%
Dorsalis pedis	102	75.55	7	46.66
Lateral plantar	26	19.66	7	46.66
Doubtful	7	5.19	1	6.66

Race (150)

	WHITE		NEGRO	
		%		%
Dorsalis pedis	57	64.00	52	85.20
Lateral plantar	27	30.00	6	9.80
Doubtful	5	5.60	3	4.90

Right and left (150)

	RIGHT		LEFT	
		%		%
Dorsalis pedis	58	75.32	51	69.86
Lateral plantar	16	20.78	17	23.85
Doubtful	3	3.90	5	6.85

Negro males, right (32) left (28)

	RIGHT		LEFT	
		%		%
Dorsalis pedis	28	87.50	23	82.14
Lateral plantar	3	9.38	3	10.72
Doubtful	1	3.12	2	7.14

An analysis of the above figures brings out the fact that this artery predominated in the male sex, the negro race, and in the right foot. In comparing the two series, we note a percentage of 86.73 in the first series (211 feet), against 72.67 in the second series (150 feet). Combining the two series, our findings are as follows:

Series compared

	1ST SERIES (211)		2ND SERIES (150)	
		%		%
Dorsalis pedis	183	86.73	109	72.67
Lateral plantar	22	10.42	33	22.00
Doubtful	6	2.85	8	5.33

Final analysis (361)

		%
Dorsalis pedis	292	80.887
Lateral plantar	55	15.235
Doubtful	14	3.878

DISCUSSION

The plantar arterial arch has been described without exception in anatomical textbooks since as early as 1812 as being formed primarily from the lateral plantar artery (Bell, 1812; Cruveilhier, 1844; Gray, '36; Cunningham, '31; Morris, '25; and Piersol, '23). The lateral plantar artery has usually been described as passing lateralward and forward to the base of the fifth metatarsal, then medially to the level of the first interosseus space, where it is said to anastamose with the deep plantar branch of the dorsalis pedis artery, forming the plantar arch. If such were the case one would expect to find the point of smallest diameter in the arch to be located between the first and second metatarsal bones (fig. 2). This condition, however, has been found by the author to apply to only a small percentage of the feet examined in the course of this investigation.

The formation of the plantar arch by the deep plantar artery has been mentioned as a variation from the normal by both Quain (1892) and Cunningham ('31). Cunningham states (page 971): "Occasionally the anterior tibial artery and its dorsalis pedis continuation are larger than normal, and the terminal part of the dorsalis pedis takes the place, more or less completely, of the lateral plantar artery."

It is of interest to note in this connection that Morris ('25) represents by diagram the plantar arch as being formed mainly by the deep plantar branch of the dorsalis pedis artery (fig. 524; after Henle, '01) rather than by the lateral plantar branch of the posterior tibial; however, this figure does not correspond with text description of the arch.

Considerable evidence supporting the importance of the dorsalis pedis artery in the formation of the plantar arch is found in the extensive work of Corsey ('13). Corsey concluded after his dissection of the feet of fifty infant cadavers that previous anatomical descriptions were applicable to only about one third of the cases examined. He found that in over half of the cases the plantar arch was formed almost entirely by the deep plantar artery while in many of the remaining cases at least a part of the interosseus arteries were derived from this artery. A somewhat larger percentage of cases was recorded by Adachi ('28), who, reporting on 125 Japanese feet, found that the plantar arch was formed by the deep plantar branch of the dorsalis pedis in 72% of his cases. Lanz and Wachsmuth derive the arch from this source in 43% of German cadavers.

Not only do the findings of the present study corroborate those of Corsey and Adachi, but the number of cases (80.887%) in which the plantar arch was formed by the deep plantar artery actually exceeds that reported by Corsey and by Adachi.

Further support of this view may be found in a consideration of the arterial circulation of the hand. Frequent comparisons have been made between the plantar arch of the foot and the deep volar arch of the hand, the major portion of which is formed by the radial artery. The radial artery on the dorsum of the hand is comparable to the dorsalis pedis on the back of the foot, particularly since each is continuous through the first interosseus space between the two heads of the first interosseus muscle to pass to the palmar and plantar surfaces respectively. The radial artery then passes from the upper end of the first interosseous space transversely across the palm to the base of the fifth metacarpal bone, where it anastomoses with the deep volar branch of the ulnar artery, completing the deep volar arch. It is the author's belief that in the same manner the deep plantar branch of the dorsalis pedis forms the major part of its corresponding arch.

Cruveilhier (1844, page 573) stated this comparison in a more definite manner as follows: ". . . the dorsal artery of the foot corresponds to the carpal portion of the radial; and the plantar arch, which is continuous with the dorsal artery of the foot, represents the deep palmar arch, which is the continuation of the radial in the hand."

In the light of such comparisons and the large number of cases in which the deep plantar artery forms the plantar arch it appears that such a condition is more than an occasional occurrence. Further investigation, which is now in progress should aid in establishing the role of the deep plantar artery in the formation of the plantar arterial arch.

SUMMARY AND CONCLUSIONS

1. In a majority of the cases (80.887%) the plantar arch was found to be formed by the deep plantar branch of the dorsalis pedis artery.
2. In 15.235% the arterial arch was formed by the lateral plantar artery.
3. There was some indication that the direction of the blood current influences the manner of origin of the plantar metatarsal arteries.

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THE EXTENT OF RECURRENT GENICULO-CALCARINE FIBERS (LOOP OF ARCHAMBAULT AND MEYER) AS DEMONSTRATED BY GROSS BRAIN DISSECTION

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TWO FIGURES

INTRODUCTION

When B. Stilling, a German surgeon-anatomist, on January 25, 1842, cut with a hand razor very thin slices from the end of a piece of spinal cord that had been frozen at —16°C. and upon examining them (unstained) under the microscope (× 15) saw transversely running (commissural) fibers, the serial section method of studying the central nervous system was born. After J. Gerlach (1858) had developed a practical way of using carmine on brain tissue (through inadvertently leaving a piece of cerebellum over night in a dilute solution of the stain), and the pathologist E. Klebs had introduced paraffin (1869) as an embedding medium (thus facilitating the use of the microtome, which could now be employed much as it had been used by botanists for more than a hundred years previous to this time), and especially after Flechsig's success (1876) in working out fiber tracts by studying the time at which they become myelinated, the following of fiber bundles in the central nervous system was done almost wholly by means of serial sections. The appearance of Weigert's superb myelin sheath stain in 1884 seemed to sound the final death knell of studying nerve fasciculi by actual dissection such as had been in vogue especially since late in the eighteenth century.

Before biologists thought of preliminary hardening of the brain, very little was known about the arrangement of the fibers in the white matter. Brain dissection for 2000 years had been confined essentially to exposing and exploring the ventricles. Vieussens (1641–1716) of France had made some headway on fresh brains for he showed in his Neurographia (1685) some continuity of the corona radiata, internal capsule, cerebral peduncles, pyramidal fasciculi of the pons and medulla oblongata. One of the first to harden the brain for dissection purposes was the great French physician and comparative anatomist Vicq d'Azyr (1748–1794), who used alcohol to which had been added salt petre or hydrochloric acid. This enabled him to dissect out additional bundles of fibers, among them being the mammillo-thalamic fasciculus. Reil (1759–1813) of Germany was more successful. He first hardened the brain in alcohol, then softened and darkened the gray matter by soaking the specimen several days in a solution of potassium or sodium carbonate. If necessary the brain was returned to the alcoholic bath to toughen the fibers. By stripping off fibers, layer after layer, in the direction they seem to course, he very definitely followed the cerebral peduncles down to the pyramidal decussation and up through the internal capsule to their interlacement with the corpus callosum and on to the corona radiata. The lateral lemniscus, ansa lenticularis and other fiber bundles were displayed. In 1805 at Halle he saw Gall (1758–1828), the founder of phrenology, demonstrate the internal structure of the brain by this general method. It is not clear how much technique one got from the other; but Reil was greatly impressed by Gall's dissections. Spurzheim (1776–1832), who collaborated with Gall for 13 years, claims that Gall did not commence to dissect the brain till 1800 — the year he came to study with Gall. This method of brain dissection was rapidly adopted and reached a high degree of development in the hands of H. Mayo (1796–1852) of England, Foville (1799–1878) and Gratiolet (1815–1865) of France, and F. Arnold (1803–1890) of Germany. The optic radiations have been associated with the name of Gratiolet ever since. Subsequently only sporatic use was made of this teasing method. However, Meynert (1833–1892) of Germany continued to use it. Very striking results were obtained by J. Stilling (1842–1915), an ophthalmologist of Strassburg, in 1882 on the visual pathways. He fixed the brain in a mixture of potassium dichromate and sodium sulphate, followed by alcohol, creosote and acetic acid. The method has been used and refined to the greatest extent by Clason, from about 1880 to 1910, and since then by his successor Hultkranz ('35) of Uppsala, Sweden. Attention was again called to its usefulness in 1909 by Jamieson of Edinburgh, Hoeve of Drake University and Curran of Harvard (the report of the latter two being called forth by the publication of a more general outline on brain dissection based on functional systems, by Johnston ('08) of the University of Minnesota where it has been further elaborated and continued as regular laboratory exercises for students). Its application to the study of the cerebellar peduncles and adjacent cranial nerves is found in the work of Büttner ('27) of Rostock and of the writer (Rasmussen, '40).

While now almost a lost art and superseded by reconstruction from serial sections, gross dissection of fiber bundles is of much value and can be carried out to a surprising degree on brain hardened in formalin (introduced as a fixing agent in 1893 independently by Blum and Herman). The value of this teasing method lies not only in bringing out many things more clearly in their three dimensions and topographic relations, but also in giving visual reality to what has to be accepted too often on faith.

LITERATURE ON THE RECURRENT GENICULO-CALCARINE FIBERS OF ARCHAMBAULT (TEMPORAL GENU, MEYER'S LOOP OR VENTRAL BRANCH OF OPTIC RADIATIONS)

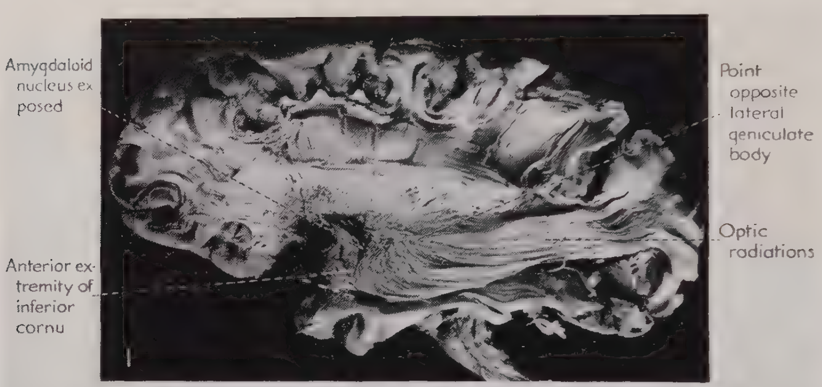
In the minds of some there is still a question as to the extent and incidence of the recurrent part of the optic radiation as they pass over the inferior cornu of the lateral ventricle. Indiscriminate dissection of this region frequently fails to give any evidence of much forward looping of the fibers. This feature of the visual pathway was very strikingly illustrated in two brains with extensive atrophy of the temporal lobe and occipital region, but with sparring of the optic radiations, which were described by Meyer ('07). In these cases the visual fibers stood out by themselves and clearly demonstrated an extensive forward detour of the ventral portion of the geniculo-calcarine tract. Glass models were reconstructed and from these Cushing ('22) had drawings made from various views which have been extensively copied. That there is some "detouring" of fibers which he considered to be more specifically geniculo-calcarine, was noted slightly earlier (1906) by Archambault ('09) as the result of studying serial sections of numerous cases of cerebral softening. Meyer called attention to the fact that a "Temporales Knie der Sehstrahlung" had already been noted by Flechsig in his myelination studies. In one of his latest publications Flechsig ('20) again shows this temporal genu in a horizontal Weigert-stained section of the brain of a 16-day-old infant; but this does not give an adequate idea of the course of these fibers. In the myelogenic studies of Pfeifer ('25) a very extensive recurrent loop was reported, which was confirmed also by Elze ('29) by gross dissection, but he illustrates his results diagrammatically by projecting them upon the reconstruction of Pfeifer. On looking back to see what earlier dissections of this region had yielded, it was found that Meynert (1884) illustrated considerable forward looping and in the text the fibers involved are regarded as part of Gratiolet's (optic) radiations. In short, the impression one gains from the literature (well reviewed also by Mayer, '40) is that part of the optic radiations swings forward virtually as far as the anterior extremity of the inferior cornu of the lateral ventricle before turning back. The applied aspect of this anatomy is evident since visual fibers may become involved in lesions that are well forward in the temporal lobe — a point abundantly emphasized especially by Cushing. Sanford and Blair ('39) found that homonymous quadrantic anopia was produced more frequently by tumors in the anterior part of the temporal lobe than by tumors located farther posteriorly.

According to an abstract of a microscopic study just published by Papez ('42) the ventral part of the optic radiations of monkeys and chimpanzees takes the same recurrent course, as was well shown in the ape in 1926 by Putnam ('26). This latest report also involved a microscopic study of fourteen human brains with various types of lesions, which further confirms the finding of Meyer, Pfeifer and Elze in general as well as the further course of these fibers after they turn posteriorly to end in the lingual gyrus. Theoretically, therefore, the visual defects from lesions involving the optic radiations in the anterior part of the temporal lobe should be predominantly in the upper field of visions; but this is not so clear-cut clinically.

GROSS DISSECTION OF RECURRENT GENICULO-CALCARINE FIBERS IN NORMAL ADULT HUMAN BRAINS

After routine dissection of the association bundles, removal of the lenticular nucleus and displaying the internal capsule from the lateral approach, further dissection of the sublenticular fibers by gently stroking forward and laterally in a curved direction, regularly brings out at least some forward curvature of fibers that pass out laterally and then backward to become a part of the optic radiations (using this term in its broad sense). With more careful forward exploration a thin layer of white matter is found whose fibers take a greater and greater recurrent direction, some of them reaching the lateral surface of the amygdaloid nucleus, as shown in figure 1. On that figure the point directly lateral to the lateral geniculate body is indicated. The degree to which these optic fibers loop forward is thus evident. They come close to the anterior commissure, as it spreads out in the temporal lobe, and can generally be demonstrated grossly almost as far anteriorly as the extreme tip of the inferior cornu of the lateral ventricle, in the roof and lateral wall of which they are located. They are separated from the ependyma by a thin stratum of corpus callosal fibers (tapetum) which run at a slight angle to the optic fibers. More posteriorly the fibers of the corpus callosum are more prominent and course at a much greater angle to the direction of the optic fibers. Anteriorly the intervening tapetum forms a thinner and thinner layer and appears to ultimately thin out to nothing. In some specimens the looping fibers are not conspicuous and are difficult or even impossible to display very clearly, apparently because of their attenuated character and great admixture with association and other projection fibers.

By removing the lower part of the temporal lobe and the floor of the inferior cornu of the ventricle, slight dissection in


Amygdaloid nucleus exposed
Anterior extremity of inferior cornu
Point opposite lateral geniculate body
Optic radiations

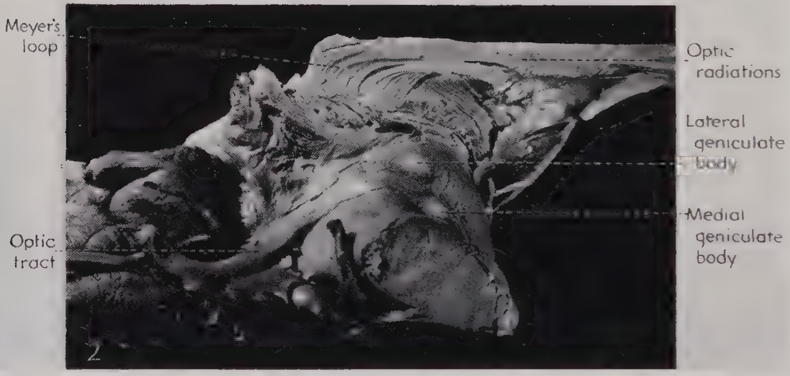

Meyer's loop
Optic tract
2
Optic radiations
Lateral geniculate body
Medial geniculate body

Fig. 1 Dissection of the geniculo-calcarine fibers (optic radiations) from the lateral exposure. All the association fibers in the upper and lateral part of the temporal lobe, as well as much of the lateral part of the frontal, parietal and occipital lobes, have been dissected away. Normal adult human brain. Hardened in formalin. ½ natural size.

Fig. 2 Dissection of the geniculo-calcarine fibers from below, by removal of the lower part of the temporal lobe, exposing the inferior cornu of the lateral ventrical and dissecting off the ependyma and thin layer of corpus callosum (tapetum), which is most evident posteriorly. Natural size, as here reproduced, and thus shows the actual distance some of the optic fibers loop forward.

the roof of the ventricle (removal of the ependyma and the few corpus callosal fibers mentioned above) yields a specimen like the one shown in figure 2. As reproduced this figure is natural size and hence shows that some geniculo-calcarine fibers may proceed fully 2 cm. forward before turning back. That they turn backward and join the compact external sagittal stratum of the optic radiations, which course toward the visual cortex, is definitely demonstrable by such dissections.

In figure 4 of Ranson's paper on dissections of the internal capsule, corona radiata and thalamic radiations, it appears that part of what is there termed fasciculus temporothalamicus or fasciculus of Arnold (1838) and fasciculus thalamotemporalis arcuatus is actually recurrent geniculo-calcarine fibers.

SUMMARY

The history and survival of gross brain dissection by teasing out fiber bundles are reviewed and the usefulness of the technique is emphasized regardless of its having fallen into disuse and been largely superseded by the serial-section method.

The majority of human brains which have been well fixed in formalin can be so dissected that fibers, apparently originating from the region of the lateral geniculate body, can be followed forward and laterally over the inferior cornu of the lateral ventricle (frequently almost to its very tip) and then backward into the lower or horizontal part of the optic radiations, which course medially under the posterior cornu of the lateral ventricle on approaching the visual cortex. It appears, therefore, that a considerable number of geniculo-calcarine fibers are involved in the loop. Fibers going forward as far as 2 cm. are generally evident in gross dissection. The most attenuated portion and scattered fibers may go even farther forward before turning back.

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THE BLASTOCYST OF THE FISHER

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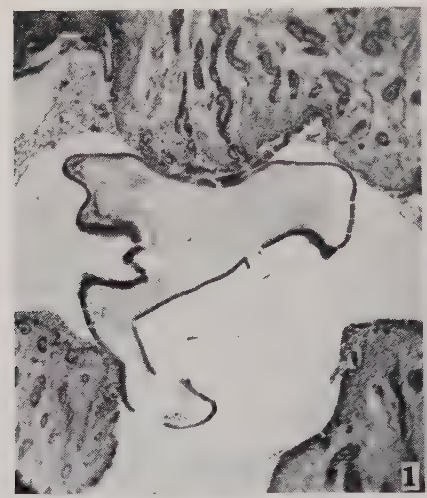
TWO FIGURES

The fisher or pecan, Martes pennanti, breeds a short time after parturition, which may occur from late March to May, and carries the young for about 51 weeks (Laberee, '41; Hall, '42). While this is by no means the longest gestation period known in mammals, it is very long for so small an animal and for a mustelid. Recent work indicates that the long gestation periods observed in some weasels, Mustela frenata and M. cicognani, and in the marten, Martes americana, is correlated with delayed implantation (Wright, '42; Marshall and Enders, '42). In these three animals as well as in the badgers Meles and Taxidea (Hamlett, '35) the fertilized ova develop to the blastocyst stage and remain in this condition for several months. Through the kindness of Dr. L. Butler of the Hudson's Bay Company, the reproductive tracts of three fishers were obtained. The ovaries of all three contained corpora lutea, so the uteri were sectioned serially and six blastocysts found.

The material sectioned included tracts from animals killed in January, February and March. The uteri of the two animals killed in January and February contained unimplanted blastocysts, but none were found in the specimen killed March 18th, although one small corpus luteum was present in one ovary. The ovaries, follicles, and oviducts of this animal showed considerable enlargement indicating the approach of estrum. The specimen secured in January contained five corpora lutea and three blastocysts. In the February specimens there were three corpora lutea and three blastocysts.

The blastocysts are similar in general appearance to those of the marten, although slightly larger. They are of a very irregular shape, probably as a result of collapse of the blastocysts during histological preparation. The most conspicuous feature is the thick surrounding zona pellucida which averages 14.4 micra in thickness. This is considerably thicker than in the marten, weasel or badger. The inner cell mass is less compact than in the marten, and is composed of a greater number of cells. The inner cell mass of two of the blastocysts from the January specimen contained 798 and 807 nuclei. This compares with about 350 in the marten, 150 in the weasel, and 50 in the badger. Preservation was not adequate to allow an accurate nuclear count to be made on all of the blastocysts preserved in February, but a single count of 844 was possible. These later blastocysts appear to be slightly more advanced than the January specimens, but there is no indication that implantation is imminent.



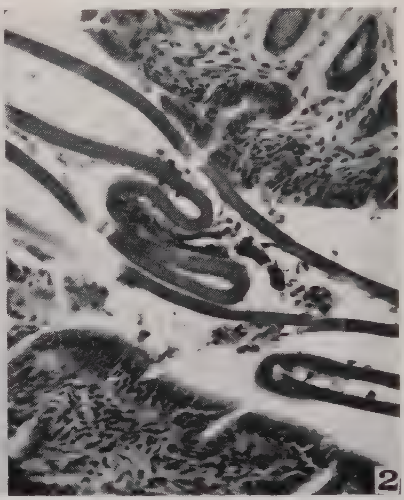


Fig. 1 Blastocyst of fisher. February. × 45.

Fig. 2 Detail of another blastocyst showing part of inner cell mass. February. × 200.

Since blastocysts are found in January and February following copulation the previous April or May, and since there is no evidence of delayed ovulation or fertilization in the mustelids, it seems probable that in the fisher unimplanted blastocysts exist from April or May until the following spring, a period of 9 or more months.

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Because of transportation and other difficulties arising as a result of the war, the fifty-ninth annual meeting of the Association which was to have been held at McGill University, Montreal, has been postponed.

In view of the circumstances we offer no apologies for the relatively small number of abstracts submitted. It is quite remarkable that any were received and we publish them in gratitude that we live in a country where we can still work, and write, and speak, and think, as we please.

ABSTRACTS

ABELL. *Vascular reactions to histamine as seen with the microscope in living, unanaesthetized rabbits.* Richard G. Abell, Department of Anatomy, University of Pennsylvania Medical School.

In 1929, Dale suggested that histamine, released by the tissues themselves, might be responsible for anaphylactic shock.

Following intravenous injections of 0.1, 0.5 and 1.0 mgm. of histamine, arterioles having control diameters up to 48 micra were observed in moat chambers (Abell and Clark, '32) in rabbits' ears to dilate approximately 50%. Temporary sticking of leukocytes to the walls of the vessels, to phase 3 of the Clark and Clark ('35) scale, was seen.

When histamine, in concentrations of from 0.05 to 0.5% in mammalian Ringer was placed upon holes in the tops of chambers, again phase 3 sticking of leukocytes occurred.

When 0.0009% or 0.005% histamine in mammalian Ringer was introduced into the reservoirs of moat chambers and left there, arterioles having diameters up to

20 micra (the largest studied) dilated 20 to 60%. A slight increase in diameter of some of the capillaries and venules occurred. Solutions of 0.05% and 1.0%, applied similarly, caused in addition extensive phase 4 sticking and massive emigration of leukocytes. Many of the capillaries became plugged with leukocytic masses.

Thus massive doses of histamine caused leukocytic sticking and emigration similar to that observed in anaphylactic shock (Abell and Schenck, '37). Histamine, however, in the concentrations used, caused dilatation of small arterioles whereas in anaphylaxis such arterioles were observed to constrict.

ABELL, R. G., see Page et al.

ADDISON. *The hypophysis of the goose-fish (Lophius pisc. L.).* William H. F. Addison, Department of Anatomy, University of Pennsylvania.

The hypophysis of Lophius lies rostral to the brain and is connected to it by a narrow stalk 10–15 mm. long. The stalk is solid and there is no hypophyseal cavity. The hypophysis is roughly globular in shape and has a depression on the dorsal surface where the stalk is attached. Sections show four constituent parts, three epithelial glandular and the neural part. The latter is the direct continuation of the stalk and soon divides into small branches in the interior of the organ. The epithelial parts are, (1) a dorso-rostral part which extends dorsally as far as the rostral side of the stalk, (2) a caudal part which surrounds branches of the pars nervosa, pars intermedia, and (3) the intervening part, the so-called transitional part (also pars distalis, anterior propria). The last consists of deeply staining cells, both acidophilic and basophilic. There are several variants of the latter and there are also groups of very small cells quite different from the rest. The pars intermedia is divided into rounded groups and strands of cells by the admixture of the neural part. The cells are pale-staining and are mostly acidophilic and basophilic with some small groups of chromophobes. There is some intermingling of the transitional part and the pars intermedia. The dorso-rostral part is variable in its staining properties and shows two arrangements of its cells in different specimens, (a) cells arranged in an open network and (b) in a more continuous formation. The material was collected during several summers at Sandwich and Woods Hole, Mass.

ALBERS, V. M., see Stowell et al.

ALDEN. *The utero-tubal junction in the albino rat.* Roland H. Alden, Osborn Zoological Laboratory, Yale University and Division of Anatomy, University of Tennessee College of Medicine. (Introduced by K. B. Corbin.)

The caudal opening of the rat oviduct lies somewhat below the apex of the uterine cornu, at the tip of a prominent papilla (Colliculus tubaricus Fischel). Instead of numerous mucosal folds there tends to be a single large one which forms from one wall of the tube, near its exit, and frequently, by fusion with the opposite wall, effects a division of the opening.

Measurements were made of the pressure necessary to force normal saline through the utero-tubal junction (from uterus to oviduct) on each side of eighteen anesthetized animals. While there was considerable variation in the pressure necessary to force the junction, in twenty measurements on ten animals in proestrus and diestrus it was, comparatively, very high (average 51 cm. mercury). With one exception, eight animals in estrus and two in early stages of pregnancy successfully resisted pressures of 100 cm. mercury.

It is difficult logically to account for the variation in resistance offered by the valve on the basis of mechanical obstruction from extensive mucosal folds and increased intrauterine pressure; no cyclic change in the character of the junction is evident upon histological examination or in study of a Born model. Some evidence suggests that proper tactile stimulation of the oviduct causes relaxation of the junction while chemical irritants produce the opposite result.

ALLEN, E., et al. *Mitotic activity in genital tissues of the mouse during pregnancy.* Edgar Allen and Richard Worthington, Department of Anatomy, Yale University.

With the colchicine technique search was made for cell division in genital tissues as one index of hormonal stimulation.

Mitosis in follicles of ovulation continues among both follicular cells and theca for a day after ovulation, even among cells extruded with ova. Mitosis in developing corpora lutea is infrequent after the third day. Medium sized follicles are subject to considerable mitotic activity during pregnancy. The inhibition which pregnancy places on their advanced development is apparent in scarcity of mitoses in outer granulosa and theca.

Vaginal and cervical epithelium, without much mitotic activity, develops superficial mucous layers. From the 12th to 17th days, however, increased mitosis accompanies extensive folding of this tissue without increasing its thickness.

Uterine epithelium, including glands, so responsive mitotically to estrogen, has no mitotic activity during the greater part of gestation. Waves of mitosis begin in the subepithelial stroma on the 4th day, radiate toward the muscle and progressively transform a loose packed connective tissue into dense decidua by the 7th day.

As the early decidua becomes vascularized intense mitotic activity appears in the sinusoidal endothelium. Chorionic villi grow with even greater rapidity, but mitoses are scarce in the placenta after the 16th day.

Uterine muscle has many cells in mitosis between the 6th and 14th days of pregnancy.

Waves of local proliferation in embryonic tissues are demonstrated by the colchicine method.

ALLEN, E., see Atkinson

ALLEN, L., et al. *Hypertrophy of the lymphatics of the greater omentum.* Lane Allen, Department of Anatomy, and Richard Torpin, Department of Gynecology, University of Georgia School of Medicine.

Three specimens have been found which demonstrate the presence and configuration of the lymphatics of the greater omentum. All consist of omenta excised at operation for removal of massive fibroid uteri.

The lymphatics of the greater omentum consist of from two to six linear plexuses which toward the distal border of the omentum are connected by lymphatic arcades formed by a single lymphatic channel. The convexity of each arcade receives a small lymphatic which arises at the distal border of the omentum. The large linear plexuses (six in one specimen, three in one specimen, two in one specimen) consist of from one to four anastomosing channels which in the specimens in some cases formed a mass as large as a man's thumb. This hypertrophy was not accompanied by hyperplasia.

All the omenta being adherent in two or more places to the fibroid uteri, the possibility was considered that the hypertrophy resulted from anastomoses between uterine and omental lymphatics. Retro-injection, which was possible due to the loss of patentcy of lymphatic valves, failed to demonstrate such connections.

Each group of lymphatics was provided with a mesentery and special blood supply. Injection with india ink disclosed a rich capillary plexus in the lymphatic valves except for a narrow clear crescent at the free margin of the valve.

ANDREW. *The true nature of so-called ''adipose tissue invasion'' within the lobules of the human pancreas in senility and in pathological conditions.* Warren Andrew, Department of Anatomy, Baylor University College of Medicine.[1]

A study of the human pancreas has revealed a frequent occurrence of spheroidal spaces among the alveoli, usually described as ''adipose tissue invasion,'' among senile persons. Fat stains show these spaces generally to lack fat. They often are lined with a simple squamous epithelium.

More important than these findings, however, is the presence of relatively enormous irregular cavities in the pancreas of senile persons. Some of these measure a millimeter in diameter. These cavities can be demonstrated as arising by a gradual confluence of the smaller spheroidal spaces.

These findings are clarified by a study of the aging pancreas in Wistar Institute rats. In these animals there is in senility a marked proliferation of duct cells in many lobules. While in man the proliferative action is less marked, the duct cells acquire expanding lumina in both forms. In the rat many lobules thus come to consist only of spheroidal or polyhedral spaces, lined with simple squamous epithelium.

There occurs in both man and rat, then, a progressive, destructive process in the pancreas in old age.

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[1] The study of Wistar Institute rats is part of a program on aging organized by Dr. E. J. Farris of The Wistar Institute, with the support of the Samuel S. Fels Fund. The author wishes to acknowledge support of his individual project by grants from the John and Mary R. Markle and the Ella Sachs Plotz Foundations.

ANDREW. *Senile changes in the pancreas of Wistar Institute rats and of man.* Warren Andrew, Department of Anatomy, Baylor University College of Medicine.[1]

A study of the pancreas of sixty Wistar Institute rats, ranging from youth through extreme senility, and of forty human subjects has been made. In early senile stages in rats there are seen degenerative alterations in the alveolar cells. The nuclei come to present great variation, in contrast to their uniformity in younger animals.

The changes in the alveoli are accompanied by a proliferation of duct cells. Masses of such cells grow in among the alveoli. In older animals these cell-groups acquire lumina. The sac-like spaces expand until they break into one another to form relatively large cavities.

In the human material the pathological conditions make it more difficult to interpret findings. The individuals over 70 years old, however, show almost without exception, numerous large, irregular cavities in the tissue, apparently formed by a confluence of spheroidal spaces. These we believe we have demonstrated to be formed by duct cells, in a process similar to that seen in the rat.

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[1] The study of Wistar Institute rats is part of a program on aging organized by Dr. E. J. Farris of The Wistar Institute, with the support of the Samuel S. Fels Fund. The author wishes to acknowledge support of his individual project by grants from the John and Mary R. Markle and the Ella Sachs Plotz Foundations.

ANSON, et al. *Stapes, fissula ante fenestram and associated structures in man: V. From the fetus of 160 mm. to term.* Barry J. Anson and Earl W. Cauldwell, Department of Anatomy, Northwestern University Medical School.

Previous studies traced the development of these structures from the 6.7 mm. to the 150 mm. stages (CR length).

A solitary basal osteogenetic center appears in the 150 mm. fetus. In successive developmental stages it encroaches on posterior crus, anterior crus, and neck, in that order; no secondary centers of ossification occur. Progressive invasion (180 mm., 183 mm., 190 mm.) results in destruction of cartilage, with the exception of articular surfaces, and replacement by bone; the periosteal shell is filled with intra- and endochondral bone and primitive marrow. The internal bony wall of crura, base and head are then entirely removed (205 mm., 210 mm., 240 mm.), leaving a flattened bilaminar basal plate, guttered crura, and excavated cylindrical neck; marrow is completely absorbed, except in the neck. At 275 mm. the capital extremity is still massive, but active osteoclasis reduces its bulk to that of the "adult" pattern, observed at 290 mm. By 345 mm. the stapes is virtually indistinguishable from that of the adult.

Like the articular surfaces of the stapes, the fissula ante fenestram represents an area of retarded histogenesis. Hyalin capsular cartilage envelops the fibrous fissula until the 210 mm. stage; thereafter, the fissula itself is chondrified and persists indefinitely as an unstable zone of cartilage in the petrous bone.

The osseous structure of the stapes is identical with that of the otic capsule.

ANSON, et al. *The deep communications of the renal veins.* Barry J. Anson, Earl W. Cauldwell and James W. Pick, Department of Anatomy, Northwestern University Medical School.

Profound dissimilarity exists between the renal venous patterns of right and left sides (in 425 cadavers); although serially arranged renal veins occasionally occur on either side, other veins are associated with the left side almost exclusively, and are not only suprarenal, gonadal, and ureteric, but also phrenic, capsular, intercostal, lumbar and iliac. In this way the renal venous drainage is brought into communication with veins of the pelvis, abdomen and thorax, and with those of the body wall.

Renal venous relationships are widely different on the two sides; not only do some veins of the left side pass dorsal to the aorta in coursing from kidney to inferior vena cava, but they may form elements in a retroaortic plexus or circumaortic venous ring, or both, and receive numerous parietal or visceral tributaries.

Rarely are the left renal veins independent of veins from neighboring parietal structures; they regularly receive the inferior phrenic vein, directly or in company with the suprarenal, upper lumbar veins directly and several lumbars via the retroaortic venous plexus, lower lumbar and iliac veins via a persistent left inferior vena cava, and venous tributaries from perinephric and general retroperitoneal tissues. They are directly associated with drainage of neighboring viscera; suprarenal gland, testis, and in the presence of a left cava, with the pelvic organs.

ANSON, B. J., see Cauldwell

AREY. *On the "crypts" of the pharygeal tonsil.* L. B. Arey, Department of Anatomy, Northwestern University.

A difference of opinion exists concerning the morphology of the pharyngeal tonsil. Many writers hold that the palatine, lingual and pharyngeal tonsils are all essentially alike in structure. Others stress the peculiar mucosal folding in the pharyngeal tonsil but deny crypts to it. However, up to the present time the exact morphology of the pharyngeal tonsil has not been determined through the method of reconstruction, as have the palatine tonsil (Minear, Arey and Milton) and the lingual tonsil (Hellman).

A pharyngeal tonsil of a newborn has been studied and reconstructed. A distinctive feature is the folding of the mucosa into roughly parallel ridges and troughs. Also prominent are the numerous pores that open from the free surface of the ridges and troughs into relatively large and long epithelial funnels. The latter are embedded in lymphoid tissue. These unbranching funnels resemble somewhat the simplest crypts of the palatine tonsil, but, unlike the palatine tonsillar crypts, each usually communicates with subtonsillar glands. Closer is the resemblance to the simple pits of the lingual glands, which do have glands opening from their depths. It might be argued whether the funnels of the pharyngeal tonsil are the dilated ducts of glands or whether they are simple crypts that have budded off glands. In either case the morphological appearance and lymphoid relationship is crypt-like in the accepted tonsillar sense.

AREY, et al. *The Huber six-somite human embryo (M 71).* L. B. Arey and J. W. Henderson, Department of Anatomy, Northwestern University.

This specimen is important because it will fill a gap in the published descriptions of human embryos. Only the fragmentary description of Klb (5–6 somites) exists between the 4-somite specimens of Bartelmez, Sternberg and Orts Llorca and the 7-somite specimen of Payne.

Detailed studies have been carried out on M 71 and extended comparisons with all published specimens between 4 and 8 somites have been made. In most respects M 71 occupies the position in the series one would expect. Among its anatomical features (and the conclusions drawn from them) the most notable are the following: It is the oldest described specimen that still presents a chordal canal in the rostral end of the head process. It is the youngest in which the rostral neural crest has been identified. It is the earliest to show an indication of the spinal neural crest. The heart should occupy the position in the Davis series now accorded by him to Klb, whereas the latter, perhaps, had better be dropped from any series depicting normal cardiac development. The level of closure of the neural folds is normally variable and a marked midbody flexure is not necessarily associated with irregularities in closure. It is more probable, rather, that the position and extent of neural tube closure, which resists flexing, influences the site of angulation.

ATKINSON. *Experiments on the maintenance of deciduomata in the mouse.* William B. Atkinson, Department of Anatomy, Yale University School of Medicine. (Introduced by Edgar Allen.)

Deciduomata produced by traumatizing the uteri of mice on the third day of pseudopregnancy regress completely within five days. Subcutaneous administration of 1 mg. of progesterone daily beginning at trauma greatly prolongs the survival of deciduomata in pseudopregnant mice, although necrosis sets in after ten days. In studying the hormonal factors involved in maintenance of deciduomata, the uteri of ovariectomized mice receiving 1 mg. of progesterone daily have been traumatized on the third day of treatment. The resulting deciduomata were maintained in a healthy state for ten days, after which degeneration was rapid despite continued treatment. Supplementing this daily dose of progesterone with 0.5 μg. of estradiol benzoate or 10 μg. of testosterone propionate daily beginning three days after traumatization, did not prolong the life of the deciduomata. In comparable experiments supplements of 10 μg. of estradiol benzoate or 250 μg. of testosterone propionate did not accelerate the onset or process of involution. It appears, therefore, that deciduomata in the mouse have an inherent life-span which is realized only if progestin is available, and neither estrogen nor androgen have modified that life-span.

ATKINSON, et al. *The production of ovarian hormones throughout pseudopregnancy in the mouse.* William B. Atkinson and Charles W. Hooker, Department of Anatomy, Yale University School of Medicine.

An attempt has been made to estimate the sequences in the production of ovarian hormones throughout pseudopregnancy in the mouse, using the changes in endometrial histology as an index. In response to estrogen the uterine epithelium becomes tall and granular and edema develops in the endometrium; in response to progestin the stromal nuclei became enlarged, vesicular, and spherical. On the day following mating there was evidence of a fairly high level of estrogen and a low-grade response to progestin. Thereafter the estrogen level rapidly decreased, reaching a minimum on the third day of pseudopregnancy. This minimum persisted until the tenth day when it rose abruptly and conspicuously, with a return of estrus on the eleventh day. The progestin level was possibly decreased on the second day of pseudopregnancy, and then increased progressively to a maximum on the seventh day. Thereafter progestin decreased gradually to the level present at time of mating.

BAILEY, et al. *Functional organization of the medial aspect of the primate cortex.* Percival Bailey, Gerhardt von Bonin, Edward W. Davis, Hugh W. Garol, Warren S. McCulloch, Ephraim Roseman, Anibal Silveira, Department of Anatomy and Research Laboratory of the Neuropsychiatric Institute, University of Illinois, College of Medicine.

By local strychninization and recording of electrical activity the medial aspect of the hemisphere of monkeys and chimpanzees has been analyzed, disclosing the medial extension of areas previously identified on the lateral side and certain new areas corresponding approximately to the cytoarchitectonic areas 31, 32, 24, 23 and 26 as given by Brodmann for the human brain. Of these, area 31 or areas 31 and 32 constitute a narrow strip running along the sulcus callosomarginalis from which it extends ventrally at the posterior end and dorsally at the anterior end. Strychninization of any part of it fires all of it. Ventral to its anterior half lies area 24 from which suppression of electrical activity and suppression of motor response are elicitable. It and all other areas yielding suppression fire into the narrow strip mentioned above. Posterior to 24 and ventral to 31 lies area 23 which fires itself and areas 18 and 19. Between this and the corpus callosum is a band perhaps a millimeter wide which fires no other region and fires itself only extremely locally. Its cytoarchitectonic identity is uncertain, but it is tentatively called area 26.

BARNES. *The ascending auditory pathway in the brain stem of the monkey.* W. T. Barnes, Institute of Neurology, Northwestern University Medical School. (Introduced by H. W. Magoun.)

Marchi studies of the brains of ten monkeys have revealed three distinct striae which originate in the cochlear nuclei and decussate in the caudal end of the pons. Some fibers enter the contralateral lateral lemniscus and continue upward to the inferior colliculus where they synapse. A few synapse in the nucleus of the lateral lemniscus. Tertiary fibers from the inferior colliculus reach the medial geniculate bodies by three routes: (1) a direct pathway from the inferior colliculus to the medial geniculate body of the same side through the inferior quadrigeminal brachium; (2) by crossing in the commissure of the inferior colliculus and continuing directly through the opposite inferior quadrigeminal brachium to the corresponding medial geniculate body; and (3) by crossing in the commissure of the inferior colliculus and, after synapsing in the opposite colliculus, fourth order neurons continue through the inferior quadrigeminal brachium to the medial geniculate body.

Other fibers of cochlear nuclei origin synapse in the contralateral superior olivary complex (probably in the homolateral complex also). From this complex tertiary neurons enter the lateral lemniscus and reach the inferior colliculus where most of them synapse. A few pass directly without synapsing into the brachium of the inferior colliculus and reach the medial geniculate body. The fibers which synapse in the inferior colliculus may reach the medial geniculate bodies by any of the three aforementioned routes.

BATES. *The origin of the hypoglossal musculature in the cat.* M. N. Bates, Laboratory of Histology and Embryology, Cornell University, and The Daniel Baugh Institute of Anatomy, Jefferson Medical College. (Introduced by J. Parsons Schaeffer.)

Two contrasting opinions concerning the source of the mammalian hypoglossal musculature — somitic origin as against ''in situ'' development — have long existed. The present study, made on the Cornell collection of cat embryos, offers evidence in a mammal that the material which furnishes the hypoglossal musculature is of somitic origin.

Diffuse cell proliferations from the ventrolateral edges of the six most anterior somites are first seen in 4½–5 mm. embryos. Converging from either direction at the level of somites 4 and 5, these streams of cells form a hypoglossal condensation. This occupies, at first, a peripheral position just under the surface ectoderm. The distal end of the material overlies the dorsolateral roof of the pericardial cavity. Additional contributions to the mass are furnished by at least two of the definitive myotomes.

Graphic reconstructions indicate that a direct correspondence exists between the number of occipital somites and hypoglossal nerve roots. The course of forward extension of the hypoglossal condensation, accompanied by nerve XII, into the definitive tongue area is traced in embryos up to the 9 mm. stage.

The changing relations of the embryo's rapidly growing pharynx are commented upon as regards the movement of this material into its ultimate position.

BATSON. *The cricopharyngeus muscle.* Oscar V. Batson, Graduate School of Medicine, University of Pennsylvania.

The lowermost fibers of the inferior constrictor muscle of the pharynx are called the cricopharyngeus muscle. These fibers, attached to the cricoid cartilage in front, form a horizontal U-shaped loop around the lower, constricted part of the pharynx. While this muscle is a definite structure to the esophagoscopist, some anatomists have denied that it functions as a barrier between the pharynx and the esophagus. The esophagoscopist must wait for it to relax before the esophagoscope can be pushed into the esophagus. In a formaldehyde hardened human cadaver this muscle has been found fixed in a state of rigor. The anatomic appearance in this specimen corroborates the clinical findings, and completely justifies the recognition of these fibers as a pharyngoesophageal sphincter. These muscle fibers seem similar to those which are responsible for the so-called hour-glass constriction of the stomach. It is possible to duplicate this human specimen in dogs by the use of strong formaldehyde solutions shortly postmortem. The functional relations between the several groups of fibers of the inferior constrictor muscle are discussed.

BECKER, R. F., see Windle et al.

BENNETT, G. A. *A morphological, experimental, and clinical study of the semitendineus muscle in various animals, including man.* George A. Bennett, The Daniel Baugh Institute of Anatomy, Jefferson Medical College.

The comparative anatomy of the tendinous inscription of the semitendineus muscle shows it to be variable in its course and position in different animals. In man it traverses the muscle obliquely and produces two muscular segments of about the same size, while in the opossum, rat, rabbit, cat, and dog, it runs more or less transversely and divides the muscle into a smaller proximal and a larger distal portion. The innervation of the muscle is such that each segment or portion formed by the inscription receives a separate branch which on stimulation results in a contraction only of its corresponding segment. The blood-supply, on the other hand, does not appear limited by the inscription and each segment in man receives blood from more than one source.

The semitendineus muscle, owing to these morphological characteristics, is structurally an ideal one for transplantation. In a case of spastic paralysis in a 12-year-old boy it was transplanted two years ago to the extensor surface of the thigh and the patella and the result has been a gratifying one.

BENNETT, G. A., et al. *Anatomical findings in a case of a persistent* right *ischiadic artery.* George A. Bennett and Harry Wilson Reed. The Daniel Baugh Institute of Anatomy, Jefferson Medical College.

The specimen was found in the right thigh of an adult male American negro during the course of routine class dissection and represents the eighteenth known case reported. The findings of interest include the area supplied by the ischiadic artery, namely, the field usually nourished by the inferior gluteal artery and the perforating branches of the profunda femoris, and the presence of a femoral artery within the range of normal size and distribution. This case tends to support Senior's contention that in the embryo the inferior gluteal artery is normally formed by the proximal end of the ischiadic.

BENNETT, H. S., see Wislocki et al.

v. BONIN. *The areal pattern of the precentral motor cortex in primates.* Gerhardt von Bonin, Department of Anatomy, University of Illinois, College of Medicine.

In both macaque and chimpanzee, the suppressor area 4 s as defined by Hines and by Dusser de Barenne and McCulloch is histologically characterized by unusually large pyramidal cells in the third layer. A similar formation is present in man and was, indeed, indicated by v. Economo. But in contrast to macaque and chimpanzee, this large-celled strip is separated from the area containing Betz cells (4 γ) by an agranular cortex (4 a). Only close to the dorsal margin of the hemisphere are the large-celled strip and the area gigantopyramidalis adjacent to each other. There is a progressive differentiation in the motor cortex as one ascends the primate scale.

Area 6 b in the macaque, the antero-ventral part of the face field in the chimpanzee and Brodmann's area 44 in man show essentially the same dysgranular type of architecture.

An area dysgranularis orbitalis is present in the macaque (where it was called area 13 by Walker), in the chimpanzee and in man where Brodmann called it 47 and v. Economo and Koskinas FFa.

The third frontal convolution is thus a very old part of the primate brain rather than a new acquisition of the human brain.

A map of the human precentral motor cortex is presented showing the areas 6 a and 6 b, 4 s, 4 a and 4 γ.

To be published in extenso in: The precentral motor cortex, edited by Paul C. Bucy. Illinois Medical and Dental Monographs.

v. BONIN, G., see Bailey et al.

BROWN. *Pigmentation of substantia nigra and locus coeruleus in certain carnivores.* James O. Brown, Department of Anatomy, University of Pennsylvania. (Introduced by Eliot R. Clark.)

Brains of three adult dogs differing widely in age and variety and of four adult cats were examined grossly and microscopically. The surface of a transversely cut fresh carnivore midbrain presents a lightly pigmented tan to reddish tan region (substantia nigra) dorsally capping the cerebral peduncle. A pale tan spot (locus coeruleus) appears at the ventrolateral corner of the central gray matter in sections through the rostral isthmus region.

Melanin occurs normally intracellularly in each region in the dog and the cat and in unstained sections it is dark brown in color in the former but slightly more yellowish in the latter animal. Silver impregnation of the argentophil melanin aids in making more readily visible intracellular pigmentation.

From the material available, pigmentation of these brain regions is a dependency of age since the amount of melanin increases in each from the young to the old individual. While a majority of neurons of substantia nigra and locus coeruleus contain pigment in varying amounts others show a complete absence of melanin.

BRUES, et al. *Cell vacuolation in high potassium media.* Austin M. Brues and Edith N. Rathbun, Medical Laboratories of the Harvard Cancer Commission, Massachusetts General Hospital.

Cultures of embryonal tissues have been grown in media in which part or all of the sodium has been replaced by equivalent amounts of potassium. Replacement of one-quarter or more of the sodium by potassium results within two hours in the development of aqueous vacuoles. These vacuoles become more prominent as more potassium is used. The effect is similar to that of aliphatic amines (Brues and Jackson, 1941) in that it fails to occur in tumor cells, is greatly enhanced by increased pH of the medium, and is wholly reversible, even from the apparently degenerated state seen in media containing no sodium. Although this vacuolation resembles that of cells deprived of glucose, the glucose metabolism of cells in high potassium media is unaltered.

Neither vacuolation nor its reversal occurs at low temperatures, suggesting that some process other than diffusion is concerned.

BURR. *Neuroid transmission in Mimosa.* H. S. Burr, Section of Neuro-Anatomy, Yale University.

Electrical correlates of a propagated impulse in Mimosa have been studied using modern methods, in a system somewhat less complex than the vertebrate nervous system. With reversible non-polarizable electrodes, a DC microvoltmeter, and a recording galvanometer, the propagated impulse is recorded as a series of electrical changes almost identical with those found in the action current potential of a nerve fiber. In young, healthy plants a stimulus is followed by a negative shift in voltage varying in amount between 50 to 100 millivolts. Within two or three seconds the potential rapidly rapidly returns to the baseline, overshooting it by some three to six millivolts and then slowly recrossing the baseline there to establish a slowly changing after potential of several millivolts. This curve has the same spike, negative after potential, and positive after potential that is found in nerve fibers. Only the magnitude and time factors are different. During the positive after potential the system is refractory. Supernormal response during the negative after potential has not yet been obtained. The system is absolutely refractory unless there is a positive-negative gradient, directed centripetally. This must be at least a millivolt per millimeter or no reaction occurs. Microscopic studies of the anatomy of the system have been included in the study.

CAMERON. *Spacing and rearrangement of glass "embryos" in the uterine horn of the albino rat.* John A. Cameron, Department of Anatomy, University of Missouri.

Near the end of the fourth day after copulation the embryos of the albino rat pass from the oviduct into the uterine horn. Within the next 12 hours or less they become spaced along the length of the horn and begin to implant. Some workers believe that the first to enter the horn implants nearest the oviduct, the second just below the first, and so on. To indicate whether the order of entrance of individual embryos is preserved or not and whether spacing changes this order, glass spheres were introduced through a large hypodermic needle into the upper ends of the uterine horns of a large series of female rats. By reference to a key color chart the changes in order of the spheres could be determined when the rat was opened 1 hour later. In the majority of cases more than half the spheres had changed their positions in the series although some cases had only a few changes of position and a few cases preserved the original order. In 3 cases spheres were rearranged and spaced between natural early implantation sites. These experiments suggest that order of entrance into the uterus does not determine the position of implantation along the uterus.

The well known precocity of the embryos furthest from the vagina may then be referred to the probable gradients of nutrition and oxygen supply rather than to the often postulated time order of implantation along the uterus.

CAREY, et al. *Morphologic effects of fatigue and anoxia on the secretion mechanism of motor end plates.* Eben J. Carey, Leo C. Massopust, Walter Zeit, John T. Schmitz, James T. Keyes, Department of Anatomy, Marquette University School of Medicine.

Chameleons, like frogs, very quickly undergo fatigue to exhaustion by either hopping or running continuously on polished floor for two to ten minutes. The chameleons have a block of neuromuscular transmission correlated morphologically by: increased amount of spherical masses and longitudinal streamers of Kühne's granules related to beaded and pleomorphic hypolemmal axons during early stages of fatigue. During late stages of fatigue, the hypo- and epilemmal axons have deeply stained spherical ''retention cysts'' with absence of secreted Kühne's granules. When chameleons are fatigued through chemical stimulation to exhaustion by sublethal dose of strychnine and then further fatigued by mechanically tapping the tail, the attenuated beaded and grape-like axons stain poorly with gold chloride and are devoid of related Kühne's secretion granules. Quick strychninization with a lethal dose gives a direct and gradual transition from hypolemmal axons to Kühne's granules with a corresponding change in intensity and color reaction with gold. This morphologic evidence supports the suggestion that the sole plate of Kühne is a granular secretion product of the hypolemmal axons.

Anoxia during early stages is accompanied by morphologic changes of motor end plates comparable to those produced by exercise fatigue. During the lethal stage of anoxia there is complete block of neuromuscular transmission and absence of Kühne's granules. The hypolemmal axons are thin, sharp, very slightly beaded, angular and pointed.

CAREY, et al. *Morphologic effects of prostigmine, quinidine and curare on the secretion mechanism of motor end plates.*[1] Eben J. Carey, Leo C. Massopust, Walter Zeit, John T. Schmitz, James T. Keyes, Department of Anatomy, Marquette University School of Medicine.

Further experimental morphologic evidence of periodic granular secretion from, and ameboid motion of, hypolemmal axons of motor nerve endings in rats and chameleons is presented in support of the chemical theory of neuromuscular transmission. Augmentation of transmission by prostigmine and other selected stimulating chemicals is correlated morphologically by: ameboid expansion of majority of beaded and grape-like hypolemmal axons; variations from normal amounts to complete absence of Kühne's granules.

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[1] Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

Blocking of neuromuscular transmission by either quinidine or curare is correlated histologically by: ameboid retraction, widening and increased gold staining of majority of hypolemmal axons; enormous augmentation of agglutinated masses and streamers of non-dispersed Kühne's granules.

When curare blocking follows prostigmine and other stimulating chemicals large ''retention cysts'' occur in some of the hypolemmal and epilemmal axons that vary from 10 to 50 microns in diameter with absence of Kühne's granules. Kühne's granules appear to be variable and periodic secretory products of the hypolemmal nerve axons which act like microscopic gland terminals in the muscle fiber. Sole plate of Kühne is not preformed and constant component of motor end plate, as now considered, in reptiles and mammals. This morphologic evidence supports the chemical theory of neuromuscular transmission and correlates the pleomorphism of motor nerve plates and periodic presence and absence of granular sole plate of Kühne with physiologic and pathologic nerve and muscle activities.

CAULDWELL. *Postrenal duplication of the inferior vena cava.* Earl W. Cauldwell, Department of Anatomy, Northwestern University Medical School. (Introduced by Barry J. Anson.)

Persistence of a left-sided longitudinal channel is often observed in association with the caval and lumbar venous systems. In a series of 250 cadavers examined, this vessel was found to extend between the renal vein (pre- or post-aortic) and the third, or the fourth, lumbar vein; between the renal and iliac veins; between the second or third lumbar and the iliac veins; and between the third lumbar and the femoral vein, without intervening iliac connections (1 case). The rudimentary left postrenal venae cavae received one or more lumbar veins from the corresponding side and communicated with the definitive right-sided vena cava by retroaortic anastomoses.

Bilateral postrenal venae cavae of equal size was studied in one instance; all left and a fourth right lumbar vein terminated in the left-sided channel; an interiliac vein of Oertel occurred.

A large left inferior vena cava, accompanied by a rudimentary right caval vessel, was also observed in one case; all left and a second right lumbar vein emptied into the left-sided channel, while the third right lumbar vein was a tributary of a persistent right postcardinal vein, which extended between the right renal and fifth lumbar veins.

Embryological interpretations and clinical implications were offered for the aberrant vascular patterns.

CAULDWELL, E. W., see Anson et al. 1 and 2

CHAMBERS. *Visceral and rage responses elicited by electrical stimulation of the interior of the cat's cerebellum.* William W. Chambers, Department of Anatomy, Vanderbilt University. (Introduced by James W. Ward.)

Sixty cycle sine-wave currents delivered through unipolar, glass insulated electrodes into the region of the nuclei fastigii and interpositus and adjacent cerebellar white matter in nine cats repeatedly caused micturition and pupillary dilatation in six animals (unanesthetized); pupillary dilatation only, in two; and defecation and urination in one. Urination thresholds compared favorably with those for cerebellar cortical points (i.e., for somatic responses), and urination usually occurred toward the end of a 4-second stimulus or several seconds thereafter. Concurrent with micturition and also elicitable at lower thresholds were growling, dilatation of the pupils and poorly directed biting movements followed by attempted escape in most of the animals. Somatic after-effects comparable to those described by Clark following stimulation of the cerebellar cortex occurred.

In four anesthetized cats (20–30 mg. nembutal per kilogram body weight) micturition occurred similarly in two instances, but at higher thresholds. Observed somatic motor responses were similar to those described by Hare, Magoun and Ranson resulting from stimulation of the same areas.

Bilateral frontal lobectomy (one animal) did not prevent the occurrence of the rage and visceral responses. Bladder pressures were recorded in this animal while anesthetized through a hypodermic needle in the bladder trigone, and increases in pressure occurred during cerebellar stimulation, graded with the stimulus and quantity of water in the bladder.

CHARIPPER, H. A., see Gordon et al.

CLARK, E. L., see Clark et al. 1 and 2

CLARK, E. R., see Brown

CLARK, E., et al. *The formation of venae comites.* Eliot R. Clark and Eleanor Linton Clark, Department of Anatomy, University of Pennsylvania.

In embryonic development, as pointed out by Mall, venae comites are with few if any exceptions secondary formations. In the typical first vascularization of organs or parts the first veins are at a distance from the arteries, either at the opposite side as in the brain, or alternating as in the lung.

Similarly, in the first vascularization of the space left in transparent chambers installed in rabbits' ears, arteries and veins alternate, and venae comites rarely occur. Here, however, as in the embryo, venae comites may develop as a secondary formation, and this process has been observed and many of the responsible factors discovered. They involve chiefly the following:

(1) the appearance secondarily of a growth-promoting medium, usually a mild inflammatory exudate, which stimulates a secondary growth of capillaries in the interstices of the original set;

(2) the existence next the artery of an unimpeded growth space;

(3) the presence on the artery of muscle layers impervious to endothelial growth;

(4) the splinting effect of the artery which, by lifting the tissue, lessens outside pressure on the veins next them, causing them to be retained, often at the expense of the original veins.

Many additional details have been observed and will be presented.

CLARK, E., et al. *Caliber changes in minute blood-vessels observed in the living mammal.* Eliot R. Clark and Eleanor Linton Clark, Department of Anatomy, University of Pennsylvania.

The behavior of small blood-vessels and their role in the control of peripheral circulation were studied microscopically in transparent chambers permanently installed in rabbits' ears. These observations corroborated earlier studies which showed that active contraction of mammalian vessels, both spontaneous and in response to stimulation, was confined to vessels with muscle and nerve supply.

In the case of regenerated vessels, some of the new arteries acquired typical contractility, while others developed it for part of their length or not at all. The time at which new arteries acquired contractility varied in different chambers and in different vessels. These differences were correlated with the ingrowth of new nerves. (Arteries having muscle but not nerves were *contractible*, as seen following violent struggling of the animal, or following the injection of a drug, such as adrenalin, which acts directly on smooth muscle.)

Caliber changes in mammalian *capillaries* and *venules* were passive and occurred secondarily, following changes in circulation and pressure caused by contraction and dilatation of supplying arteries. The slower of these caliber changes were due to growth properties and adaptability of the endothelium to increased or diminished flow.

Occasionally more rapid caliber changes occurred in arterial capillaries and in cross-connecting capillaries between veins. The former were due to the elasticity and turgor of the vessel wall, and the latter to suction.

Rapid passive dilatation of capillaries may also result from a change in endothelial consistency as in inflammation.

CLARK, G., see Rasmussen et al.

CLARK, S., et al. *The effects of rapid compression waves on animals submerged in water.* Sam L. Clark and James W. Ward, Department of Anatomy, Vanderbilt University Medical School.

One hundred and thirty-seven animals (rats, mice and cats) have been partly or completely surrounded by rapid, brief compression waves in water open to air. Following an adequate blow, lesions occurred similar to those described for animals and man exposed to high explosives in air (massive pulmonary hemorrhage, bruising or rupture of abdominal viscera). The following factors varied the effect: 1. Weight (less effect with heavier animals). 2. Stage of respiration (more damage to lungs if distended). 3. Position of animal (when doubled up the covered areas partly spared). 4. Amount of submersion (with upper thorax out of water, no hemorrhage in upper parts of lung). 5. Protective coverings (''life preservers'' of cork or sponge rubber protected from otherwise fatal blasts). Subcutaneous emphysema, air or gas in intestines and air in lungs predisposed to bruising and hemorrhage.

After the impact in anesthetized and unanesthetized animals respiration stopped a few seconds or might not be resumed following maximal blows (unless both vagi were cut and then respiration was unaffected unless death occurred within a few minutes from pulmonary hemorrhage). The corneal reflex was abolished instantly in only a few animals (even if death followed), but no other evidence of cerebral concussion occurred (except slowing of the heart in some experiments) even when the entire animal was submerged. The electrocorticogram has also failed so far to demonstrate the phenomenon of concussion. The wave producing the impact is believed to travel at about 5000 ft./sec. or more and to last about 1/1000 second or less.

CLARKE, et al. *Further studies concerning the overt and masked actions of steroids.* Eleanor Clarke and Hans Selye, Department of Anatomy, McGill University.

Experiments on spayed rats indicate that a mixture containing a constant proportion of progesterone and α-estradiol causes vaginal cornification at low, but not at high dose levels. The experiments are taken to support the concept according to which the transitory vaginal cornification elicited in the spayed rodent by all hormonally active steroids is due to the fact that these compounds contain a certain amount of folliculoid activity. This is manifest during the first few days of treatment when the hormone concentration in the body is still low but becomes masked when this concentration rises above a certain level.

CORBIN, K. B., see Alden

CORBIN, K. B., see Eliot et al.

CORBIN, K. B., see Hauser et al.

CRAIGIE. *The capillary bed in the cerebral hemispheres of certain lungfishes.* E. Horne Craigie, Department of Zoology, University of Toronto.

In Protopterus, the vascular bed appears to be composed of more or less irregular groups of wide, low arches, many of which are connected with neighboring ones so that a simple, wide-meshed reticulum exists. No evidence was observed of the peculiar capillary loops characteristic of the brains of most tailed amphibians and of gymnophiona.

In Epiceratodus, the capillaries tend to run in more irregular courses, many of them roughly perpendicular to the surface, and form a very large-meshed reticulum, though occasional vessels apparently neither branch nor anastomose within the brain tissue. Here and there occur closely associated pairs of vessels which seem to constitute loops completely comparable with those in the brains of tailed amphibians. No evidence of such loops has hitherto been found in any other true fishes.

CRAIGIE, E. H., see Glenny

CRIDER, J. O., see Ramsay et al.

CROSS. *The anatomy of the human lung root.* Glen O. Cross, Department of Anatomy, St. Louis University School of Medicine. (Introduced by D. M. Schoemaker).

Reflex bradycardia and heart block from apparent inclusion of nerves in mass ligature of the human lung root have occurred. In addition, ten recorded instances of large anomalous pulmonary arteries in the pulmonary ligaments have been accounted in the literature. With these surgical implications in mind the anatomy of the lung root has been reviewed in seventy cadavers and post-mortem bodies with the following findings: (1) Extensive sub-pleural connections from the vagus nerve and sympathetics to the pulmonary plexuses with interconnections between cardiac, pulmonary, and coronary plexuses are constant findings. (2) Venous and arterial branches in the pulmonary ligament appeared in all cases, one showing a large branch arising directly from the thoracic aorta. (3) Bronchial arteries originated only from the thoracic aorta, a few arising as right or left directly from the anterior surface. (4) No marked variations in the manner of division of the arteries, veins, or primary bronchi at the hilum were noted.

CURL, et al. *Length of inguinal canal in fetus and new-born.* Howard Curl and Robert G. Tromly, Division of Anatomy, University of Tennessee. (Introduced by F. Harrison.)

Because there is widespread belief among medical students and physicians that there is no inguinal canal at the time the testis passes from abdomen to scrotum the following study was undertaken.

The right and left inguinal region and inguinal canal were dissected in each of 25 infant and fetal cadavers, 17 male and 8 female. All material had been ''fixed'' or embalmed before dissection. In the youngest male dissected, 13.0 cm. crown-rump, there was a definite inguinal canal 4 mm. in length. In the oldest fetuses with abdominal testis, 25–26 cm. crown-rump, the canal was 9 mm. in length. The testis at this time measured approximately 3 mm. in diameter and 7 mm. in length. In the youngest fetus with a scrotal testis, 30.5 cm. crown-rump, the canal was 10 mm. in length. All infants with a scrotal testis were still-born except one which was 24 days old. In these the inguinal canal measured from 10 to 15 mm. in length. Right and left canal in each case were approximately the same length. The inguinal canal in the female was just as definite and of approximately the same length as that in the male of the same age.

DAVIS, E. W., see Bailey et al.

DEY. *Disturbances of sexual function in the female guinea pig following injury to the median eminence.* Frederick L. Dey, Institute of Neurology, Northwestern University Medical School.

Previous investigations carried out in our laboratory indicate the possibility that the median eminence is concerned in the regulation of pituitary gonadotrophic functions in the female guinea pig. In the present experiments, the effect of direct injury to this structure was studied.

Electrolytic lesions were made in the region of the median eminence in 22 female guinea pigs. Fifteen of the animals ceased running estrous cycles following this procedure. Microscopic study of the brains showed that the major portion of the median eminence was destroyed in 14 of the acyclic animals. The lesion of the exceptional animal transected the hypophysial stalk but caused only slight injury to the medial eminence itself. The lesions of the 7 animals that ran normal cycles post-operatively lay either rostral or else caudal to the median eminence.

The acyclic animals were injected with a course of ovarian hormones 50 days postoperatively. Following this procedure, 4 animals regained normal cyclic activity. The damage to the median eminence in these 4 animals was considerably less than that seen in the permanently acyclic group.

These results may be regarded as offering further support to the suggestion that the median eminence plays an important role in the control of the gonadotrophic functions of the anterior pituitary.

DOUGHERTY, et al. *Changes in the myeloid and lymphoid tissues of estrogen treated dogs.* Thomas F. Dougherty, W. Lane Williams, and W. U. Gardner, Department of Anatomy, Yale University School of Medicine.

Five spayed dogs weighing 3 to 5 kg. received daily subcutaneous injections of 1 mg. to 1.6 mg. of estrone or estradiol benzoate in sesame oil solution. A control dog received 0.6 cc. sesame oil daily.

The effect on the blood picture of the experimental dogs may be divided into two phases. During the first 5 weeks there was a gradually increasing leukocytosis, normal erythrocyte count and haemoglobin level. Subsequently there occurred a rapidly developing neutropenia, relative lymphocytosis, and corresponding fall in total erythrocyte count and haemoglobin. The animals died within 50 to 70 days after beginning of treatment. Terminally there was a distinct agranulocytosis (250–1000 cells cu. mm.) and an extreme normocytic anemia (1.5 to 2 millions RBC).

The bone marrow of the estrogen-treated dogs became gelatinous and completely aplastic. The only cells present were plasma cells, small lymphocytes, mast cells, reticular cells and macrophages.

The spleens were smaller than normal and were characterized histologically by absence of germinal centers; marked depletion of lymphocytes; large numbers of plasma cells and small normoblasts; many hemosiderin-containing macrophages; and, a proliferation of littoral reticulum.

A disappearance of germinal centers and a depletion of lymphocytes were the most marked alterations in the hemorrhagic and congested lymph-nodes. The livers were extremely congested. The Kuppfer cells were swollen and projected into distended sinusoids.

ELIOT, et al. *The number and size of muscle fibers in the rat soleus in relation to age, sex, and exercise.* T. S. Eliot, R. C. Wigginton, and K. B. Corbin, Division of Anatomy, University of Tennessee College of Medicine.

Litter mates from a genetically homogenous strain of white rats were weaned at 25 days and divided into control and experimental groups, sexes equally divided.

One group was placed in Slonaker revolving cages with counters; the others were placed in small cages in the same room and on identical diet. The animals in revolving cages exercised voluntarily. Only those maintaining daily averages in excess of one thousand revolutions were used in comparisons.

At 98 or 375 days, the animals were sacrificed. The right solei were removed from control and exercised animals and subjected to identical fixation, embedding, cutting, and staining procedures. Cross-sections through the midpoint of the muscles were used for fiber counts and area determinations. Of sixty animals, fifteen met all requirements for control and experimental groups.

Neither age nor sex influenced the number of muscle fibers, nor did sex influence the cross-sectional area of individual fibers. The cross-sectional area seemed, in some cases, related to the animal's weight, the larger animals having larger fibers; but exercised animals, regardless of body weight, tended to have fibers with larger cross-sectional areas, the ratio at 375 days being, exercised: control as 1.13: 1.00. More striking was the demonstration of precocious fiber growth in exercised animals at 98 days, the ratio of cross-sectional area being, exercised: control as 1.56: 1.00.

ENDERS, et al. *Early implantation and shortened gestation in the marten.* Robert K. Enders and Oliver P. Pearson, Swarthmore College and U. S. Department of the Interior.

The gestation period of the marten, Martes, is approximately eight months, breeding taking place in July–August and the young being born the following April. Recent work has shown that after fertilization blastocysts remain unimplanted in the uterus until after February 19.

Eight marten were bred during the normal breeding season and then by electric lighting were subjected to increasing length of day beginning September 21. This treatment induced early implantation. On December 4 marked mammary development was observed in three animals. Embryos comparable in development to those found in wild marten in late March were recovered from one of these animals. On December 20 and 23 litters were born to the other two. Neither mammary development nor palpation indicated developing embryos in the other four experimental animals.

A high percentage of wild marten caught in winter contain unimplanted blastocysts, whereas captive marten are notoriously unproductive. This low fertility may be due in part to loss before or at implantation. If this assumption is correct it is probable that illumination has not only induced early implantation but also increased the number of females in which blastocysts implanted.

EVANS, G. *Further studies on an Egyptian mummy.* Gertrude S. Evans, William Waldo Blackman Laboratory of Anatomy, New York Medical College. (Introduced by Thomas Horace Evans).

The cranial bones and hair have been studied and the shafts of the latter found to be circular in cross section. This contrasts with sections of hair from an American negress who had successfully undergone a ''hair-straightening'' treatment but in whom the sections still showed an irregular margin. It was possible to demonstrate cortex, medulla and central canal in this 3000-year-old mummy hair and there were indications that the original color was red.

EVANS, T. *A function of the papillary musculature as evidenced by study of hearts of dog, sheep and man.* Thomas Horace Evans, William Waldo Blackman Laboratory of Anatomy, New York Medical College.

The left anterior papillary muscle of the right ventricle varies in position, resulting in differences in the power exerted. In the sheep its base is usually far along the sternocostal wall of the chamber, in the dog in the septoparietal angle. On contraction the distance from implantation area to atrioventricular ring lessens and since chordae tendineae pass onto ventricular surface of cusp the latter is pulled into ventricle, opening ostium. To facilitate filling of ventricle its wall is bulged outward, aspirating the final blood at pre-systole.

Sheep and dogs have been studied at sea level and at 2600 ft. altitude. In the former marked rise in heart rate can result in breathlessness (respiratory rate approaching pulse rate), fainting and death; whereas in the human, thyrotoxicosis with its increased pulse, does not result in an increased respiration rate.

EVANS, T. *A study of some anomalous human hearts.* Thomas Horace Evans, William Waldo Blackman Laboratory of Anatomy, New York Medical College.

In an examination of 1200 human heart specimens the following interesting conditions were found:

1. Two cases in which the coronary sinus opened into the left atrium. One of these was a perfect specimen (B-697 Flower), the other was not preserved.
2. Two cases which had been dissected but showed evidence that the sinus opened not only into the right atrium but also into the left by a communicating vein.
3. The left superior vena cava was often functional, but with a small lumen.
4. In specimens showing right subclavian origin from aorta distad to the left subclavian, the bronchial arteries arose distad to region of ductus arteriosus and proximad to the right subclavian (7 cases).

The idea of coronary artery origin, and near the dorsal aorta, bronchial artery origin, as branches from an abortive seventh aortic arch is advanced; as is also the theory that fetal oxygenation may occur to some degree in pulmonic areas by interchange through the tissue between vessels carrying blood of different oxygen content.

EVANS, T. H., see Evans, G.

EVERETT. *Evidence suggesting a possible role for the lactogenic hormone in the estrous cycle of the rat.* John W. Everett, Duke University School of Medicine.

Earlier reports have shown that cyclic ovulation may be restored to persistent-estrous rats by progesterone, either in small daily injections or in single doses timed to coincide with proestrus. Astwood ('41) has pictured "luteotrophin" (= lactogen?) as the gonadotrophe responsible for progesterone secretion, first by the follicles during preovulatory swelling, later by the new corpora lutea.

Possibly persistent-estrous rats are deficient in lactogen secretion and thus fail to secrete progesterone during proestrus.

Selected DA rats in persistent estrus were treated in various ways with Prolactin (Schering). In the absence of previously formed corpora lutea this material failed to cause ovulation when injected (5 to 50 i.u. daily) late in diestrus and in early estrus. However, after new corpora lutea were first induced by progesterone, subsequent ovulatory cycles were regularly obtained by suitable manipulation of lactogen dose level: (1) Daily injection of 15 to 30 i.u. for 2 to 6 days was always followed by cyclic estrus with ovulation, about 3 days after the last injection. (2) Dose levels of 2 to 5 i.u. per day allowed the return of estrus after 3 to 6 days in spite of continued treatment. If, during proestrus, the lactogen dose was then increased to 15 i.u. or more, or even if it was maintained at 5 i.u., ovulation usually followed, closely simulating the normal cycle chronologically and histologically.

FARRIS, et al. *Effects of a neurophysiological stimulus on the breeding of albino rats.*[1] Edmond J. Farris and Eleanor H. Yeakel, The Wistar Institute of Anatomy and Biology.

A study was conducted on male and female rats of three age groups to test the effect of a neurophysiological stimulus on breeding. Experimental rats were stimulated by sound from an air blast five minutes daily for five days a week continuously since weaning and throughout this experiment. The auditory stimulation frequently caused running attacks and convulsions.

In the younger groups (74–261 days old), 69.8% of the 63 experimental females became pregnant, while 39.7% of the sixty-three controls became pregnant. None of the older animals (300–414 days) produced litters.

88.6% of the young born to experimental females survived the first day, compared with 98.5% for controls. At weaning, 77.2% of experimental young and 88.6% of control young were alive. The differences in survival at birth and at weaning were statistically significant. No significant differences were found in the weights, sex ratio, or number of young at birth.

We conclude that exposure to air blasting resulted in more pregnancies, but probably depressed maternal behavior.

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[1] Aided in part by a grant from the Samuel S. Fels Fund.

FARRIS. *Effect of estrus and ovulation upon blood counts of albino rats.*[1] Edmond J. Farris, The Wistar Institute of Anatomy and Biology.

One hundred and eleven blood counts were made on female albino rats at representative intervals during estrus and corresponding diestrum. The estrus periods were determined very accurately by running activity.

During diestrum there was a nocturnal reduction in both the total number of leucocytes and lymphocytes.

During estrus the total leucocyte count showed a marked leucopenia, much greater than the daily reduction of diestrum, the lowest count being evident four hours after the beginning of heat. The lymphocyte count paralleled closely that of the total leucocyte count.

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[1] Aided in part by a grant from the Samuel S. Fels Fund.

Shortly after ovulation a marked increase in both the total number of leucocytes and lymphocytes occurred.

It is concluded there is a daily leucocyte cycle in the female albino rat, emphasized by a marked leucopenia during estrus, and that with ovulation there is a marked increase in the total number of leucocytes and lymphocytes.

FOLEY. *Denervation experiments on the cervical sympathetic trunk.* James O. Foley, Department of Anatomy, University of Alabama.

It has been demonstrated by the writer that the cervical sympathetic trunks of some cats are devoid of nerve fibers when its preganglionic axons have been eliminated and the nodose and superior cervical ganglia have been removed. The experiments reported here show that such measures do not always denervate the trunk. Persistent axons were found in the trunks of all but one of six cats in which procedures equivalent to those described above were effected. Histological examination of the degenerated trunks which contained axons showed that variable numbers of cells (4–7858) were present. Apparently some of these cells project fibers upward in the trunk, for 100 to 2389 axons were enumerated in the cephalic ends of the innervated trunks. Confirmation of this conclusion was furnished when the cervical sympathetic trunk was completely isolated in three cats by removing the stellate, middle cervical and superior cervical ganglia. The cephalic ends of the isolated segments of the trunks of these animals contained 54, 116 and no axons while the degenerated trunks contained respectively 60, 64 and 1 cells. These observations show that the cervical sympathetic trunk of the cat contains; in addition to the nerve fibers which enter it from the vagus, superior cervical ganglion and thoracic spinal nerves; axons which are derived from cells located in the trunk between the superior and middle cervical ganglia.

FOX. *The stria terminalis, longitudinal association bundle and precommissural fornix fibers in the cat.* Clement A. Fox, Department of Anatomy, Marquette University School of Medicine.

In retrograde cell degeneration and Marchi studies of the amygdala and its connections, lesions were produced (Horsley-Clarke technique) in 11 cat and 10 kitten brains.

No retrograde cell degeneration in the amygdala followed lesions with these relationships to this complex: in the mid-course of the stria terminalis; medial to the amygdala; lateral to the amygdala; anterior to the amygdala.

Lesions interrupting the stria terminalis in mid-course indicated that all of the stria is efferent with respect to the amygdala. Lesions in the caudomedial part of the amygdala, involving particularly the basal amygdaloid nucleus, degenerated the stria terminalis and the longitudinal association bundle. Lesions in the anterior amygdaloid area and in the lateral amygdaloid nucleus did not degenerate these tracts.

The stria terminalis was traced to the preoptic region adjacent to the third ventricle. Neither a stria medullaris component nor a commissural component of the stria terminalis appeared in these Marchi preparations.

The longitudinal association bundle was followed to the stria terminalis bed nucleus in the anterior commissure region. It arises principally from the large cell portion of the basal amygdaloid nucleus.

Certain of these lesions included a small portion of the hippocampus adjacent to the amygdala, degenerating precommissural fornix fibers which proceeded in the lateral part of the fimbria fornix to the lateral septal nucleus. Some of these fibers entered the nucleus accumbens.

FOX, C. A., see Schmitz

FOX, C. A., see Magoun et al.

GARDNER, W. U., see Dougherty et al.

GAROL, H. W., see Bailey et al.

GLENNY. *Some modifications of certain embryonic vessels in birds.* Fred H. Glenny, R.C.A.M.C., formerly Department of Zoology, University of Toronto. (Introduced by E. Horne Craigie.)

During the embryonic development of birds, a series of changes involving the ductus caroticus, systemic and pulmonary arches, and the internal and external carotid arteries takes place. The left systemic arch atrophies and the left radix aortae anastomoses with the pulmonary arch proximally; the left ductus caroticus loses its connection with the left radix and becomes the functionally modified ductus shawi, receiving branches from the oesophagus, trachea, and syrinx; later the right ductus caroticus becomes similarly modified, remains patent in individual specimens, remains as the ligamentum caroticum in certain species, or becomes functionally modified as the ductus shawi but still retains a short ligamentous attachment to the right radix. The left ductus botalli atrophies and almost or completely disappears; the left radix aortae atrophies and remains as the ligamentum aortae — occasionally it retains a lumen or becomes functionally modified as a dorsal intercostal artery; the right ductus botalli becomes occluded and may or may not completely disappear. The internal and external carotids anastomose in part. In ''laevo-carotidinae'' forms, the right internal carotid becomes functionally modified posteriorly.

GORDON, et al. *Effect of sex hormones on anemia induced by hemorrhage in rats.* Albert S. Gordon and Harry A. Charipper, Department of Biology, Washington Square College of Arts & Science, New York University.

Five to 6 cc. of blood were drawn, by cardiac puncture, from adult male and female rats. The red cell counts dropped from normal values of 8.0–9.2 to 4.8–5.7 million/cu. mm. on the day following the bleeding. Half of the females and males were left untreated; the remainder of the females received daily injections of 20–200 R.U. estradiol and the other males, daily doses of 1.25–2.5 mg. testosterone.

Untreated females regained normal counts after 28–31 days, and the untreated males after 18–22 days. Counts were normal in testosterone-treated rats within 12–14 days. In this group the counts continued to rise and were 2.5–3.0 million/cu. mm. above normal after 43 days of injection. Animals receiving estradiol showed delayed red cell regeneration, normal counts not being attained after 43 days.

Hemoglobin regeneration was slightly more rapid in the androgen-influenced groups. Reticulocytosis was maintained in animals injected with male and female hormones. These elements, however, were more numerous in the androgen-treated animals. Marked vacuolization was observed in the bone marrows of rats injected for 43 days with estrogen. Marrows of testosterone-treated animals revealed a hyperplasia, greater than that present in untreated males. Vacuolization was absent.

It is concluded that androgens accelerate and estrogens inhibit the rate of red cell regeneration in hemorrhagic anemia by affecting their production in the bone marrow.

GOSS. *Skeletal muscle-fiber endings and their connective-tissue attachments.* Charles M. Goss, Department of Anatomy, University of Alabama.

Tissue was taken from the region of attachment of fourteen muscles from different parts of the body of adult rhesus monkeys. Sections were stained by the Foot and Masson modifications of the Bielschowsky technique. Blackened reticular connective tissue fibrillae (argyrophil fibrillae) were found over the ends of the muscle fibers separating them from the tendon. No instance of continuity of myofibrillae with tendon fibrillae was seen. The fiber ends are variously shaped: blunt, rounded, conical, tapering, or truncated. They are separated from the tendon by an appreciable distance in the majority of cases and at times by as much as 100 micra. Argyrophil fibrillae bridge the interval. The argyrophil fibrillae at the sides of the fiber continue over the ending to form a cap and are then prolonged into a narrow strand which joins the tendon, blending with it and becoming an integral part of it. The ends of the fibers are indented by projections of the argyrophil material in the form of delicate needles, spiral threads or vertical septa. These projections are clearly seen in longitudinal, transverse or oblique sections of fiber endings.

GOSS, C. M., see Sensenig

GRAY. *Further studies on the source of preganglionic fibers to the ciliary ganglion cells in chickens.* Mary E. Gray, Department of Anatomy, Vanderbilt University.

It was previously reported that disappearance of synaptic endings from the ciliary ganglion cells of chickens took place after section of the oculomotor nerve. Section of the trigeminal and of the ramus communicans from the trigeminal to the main short ciliary nerve did not affect synapses in the ganglion. To complete this study of the source of preganglionic fibers to the ciliary ganglion, the right superior cervical sympathetic ganglion was removed in five chickens.

After the operation the feathers on the right side of the head under all conditions remained in a more erect position than those on the left. The pupils, however, reacted equally to light and accommodation and there was no difference in eye color. This condition persisted until the autopsy 6 weeks later.

The orbital contents of two chickens were successfully prepared according to Ranson's pyridine silver method and sectioned serially. In both chickens the ciliary ganglion was normal. The zoning of the ganglion into large and small cell areas was well defined and synaptic endings were observed on both types of ganglion cells. This indicates that the only source of preganglionic fibers to the ciliary ganglion cells in the chicken is by way of the oculomotor nerve. It also seems apparent that the sympathetic division of the autonomic nervous system plays no part in the pupillary reactions of the chicken eye.

GRUENWALD. *The genesis of atresia of the esophagus combined with tracheo-esophageal fistula.* Peter Gruenwald, Department of Histology, Middlesex University School of Medicine.

A chick embryo of 5 days and 16 hours showed on serial sections the typical combination of atresia of the esophagus and tracheo-esophageal fistula, in addition to malformations of brain and eyes. The cells of esophageal and tracheal epithelium cannot be distinguished at this stage. However, the mesenchyme surrounding the tube usually described as trachea, shows a distinct difference in the structure of its ventral and dorsal portions. Along the ventral wall of the tube it has the character of the very dense tracheal wall, whereas the dorsal half shows the characteristic sharp division into a very loose inner, and a denser outer layer of esophageal mesenchyme. It can be assumed that these differentiations depend on the nature of the adjacent epithelium. This corroborates the author's earlier suggestion that the malformation in question develops by failure of the tracheal primordium to separate itself from the esophagus. The undivided tube forms what is regarded as trachea in the malformed region. Esophageal tissue is incorporated in its dorsal wall, thus connecting the upper and lower portions of the seemingly interrupted esophagus.

HAMILTON. *Evidences of marked stimulation by sex hormones in certain eunuchs, phenomena interpreted tentatively to result from changed function of the adrenal glands following castration.* James B. Hamilton, Department of Anatomy, Yale University.

Evidences of pronounced stimulation by sex hormones were observed in three men who had been castrated for 8 or more years. These include (1) active secretion by cutaneous sebaceous glands, with orange to red fluorescence (under near-ultraviolet light) of comedones over extensive areas of the face; (2) dense pubic and axillary hair, rapid growth of hairs of the beard, and development of secondary sexual hair on trunk and limbs; (3) values for 17-ketosteroids within the higher portion of the range in normal men, as assayed in urine collected for 6 days from one eunuch; (4) flushing of skin of scrotum, penis, and circumanal region; (5) extreme gynecomastia with several large, movable masses in one man.

Other notable features were (1) fluorescence of areas with apocrine glands, especially a bright, orange color in circumanal and perineal regions exceeding that observed in 100 other eunuchs and 122 normal men and women; white fluorescence of axillae present on occasions in one eunuch; (2) a protruding, taut abdomen. It is suggested that in these cases the adrenal glands may secrete large amounts of hormones (or their precursors) subsequent to castration, akin to conditions in certain castrated animals. Phenomena found with adrenocortical dysfunction in Cushing's syndrome, such as osteoporosis, stria of abdominal skin, hypertension, and glycosuria, were not detected.

HARMAN, P. J., see Solnitzky et al.

HARRISON, F., see Curl et al.

HARTROFT. *The size of pulmonic alveoli in the dog as determined from microsections, and a comparison of the same with the rhesus monkey and domestic cat.*[1] Walter Stanley Hartroft, The University of Western Ontario. (Introduced by Charles C. Macklin.)

Lungs of four large and four small dogs were prepared in the state of expansion using Bouin's picricformol solution: six by intravascular perfusion in the intact thorax, and two by controlled intrabronchial filling after collapse. The form of the alveolus and its relation to the alveolar sac in sections prepared by a standard procedure from subpleural regions of each of the seven lobes, is described. Five dimensions were selected and defined for measuring size of alveoli in terms of their outlines in the sections. From 400 camera lucida tracings per lobe (2800 per animal) of equal numbers of ''open'' and ''closed'' outlines drawn at 320 ×, the means, standard deviations, and probable errors of four diameters (Depth, Width, X and Y) and a fifth dimension (Mouth-opening of alveolus into alveolar sac), were determined for each animal; a total of 56,000 measurements on 22,400 alveoli. Final averages condensed from these are presented with the aid of graphs and tables. Possible relations of body weight, age, sex, fixation and mensuration methods to alveolar size are discussed. The average alveolar diameter (Depth, Width, X and Y average) for these eight dog lungs was approximately 10 microns less than the corresponding value for the monkey (M. Rhesus) and 50 microns less than the same figure for the domestic cat as previously reported by the author.

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[1] This work was done under a grant from the National Research Council of Canada and the Department of National Defense, Canada.

HARMAN, et al. *The comparative anatomy of the lateral geniculate complex of Cebus fatuellus and Ateles ater.* Pinckney J. Harman and Othmar Solnitzky, Georgetown University Brain Research Institute.

A comparison of the lateral geniculate complex of the platyrrhine monkeys Ateles ater and Cebus fatuellus reveals that the general characteristics and relationships of the optic tract, nucleus geniculatus lateralis, nucleus pregeniculatus and nucleus intergeniculatus are similar in the two forms; however, they differ in the following important respects: in Cebus the optic tract is less distinctly fasciculated; the shape of the nucleus geniculatus lateralis is less variable at different orocaudal levels; the four parvocellular laminae are less sharply delineated and do not all extend across the lateromedial diameter of the nucleus; and the nucleus intergeniculatus is less extensive and does not extend beyond the caudal pole of the nucleus geniculatus lateralis. On the other hand, in Cebus, the intervening fiber laminae are better developed; there is more extensive fusion between the nucleus intergeniculatus and the nucleus geniculatus lateralis; the hilus of the nucleus geniculatus lateralis is more distinct; and the nucleus intergeniculatus is more sharply subdivided into two parts on the basis of cyto- and myeloarchitecture.

Little support can be provided for the view that the lateral geniculate body of Cebus is better developed than that of Ateles. A comparison of this nature is so complicated by a lack of adequate criteria which could consistently establish one form above the other that the present authors prefer to leave this question open.

HARRIS. *An experimental analysis of the inferior mesenteric plexus in the cat.* Albert J. Harris, Department of Microanatomy, St. Louis University School of Medicine. (Introduced by Albert Kuntz.)

An experimental anatomical study of the inferior mesenteric plexus in the cat, in which the several components have been isolated by section and degeneration, has yielded the following results. Of the fibers which enter the plexus, 4000 are preganglionic components of the lumbar splanchnic and intermesenteric nerves. Of these 3000 terminate in the ganglia, and 1000 extend into the pelvis via the hypogastric nerves. 3000 are postganglionics which arise in the sympathetic trunks and converge upon the inferior mesenteric ganglia via the intermesenteric nerves. 2500 afferent fibers enter the ganglia, and 5000 extend beyond them. Preganglionic parasympathetic axons ascend in the hypogastric nerves and reach the colon via the inferior mesenteric nerves. These data indicate a preganglionic-postganglionic ratio of 1 to 3, a sensory-motor ratio of 1 to 3 and two afferent fibers distal to the ganglia for every one proximal to them. Each afferent fiber, on the average, gives off one collateral. The majority of the myelinated afferents are small (1–3 μ); some are of medium (4–6 μ) and large (7–10 μ) caliber; some are unmyelinated. The largest afferents terminate in Pacinian corpuscles in the mesocolon. Afferents to the colon are exclusively unmyelinated, whereas the myelinated afferents supply the pelvis via the hypogastric nerves. The preganglionic sympathetics include both unmyelinated, and medium and small myelinated fibers.

HAUSER, et al. *Virus tracing of the ophthalmic division within the spinal tract of the fifth nerve.* Fred W. Hauser and Kendall B. Corbin, Division of Anatomy, University of Tennessee College of Medicine.

The HF strain of herpes simplex virus was inoculated onto the scarified right corneae in adult rabbits. After from 5 to 7 days the animals were fixed by injection with Zenker's acetic fluid, the brain stems serially sectioned after paraffin embedding, and the sections stained with H. and E.

The path of the virus could be followed by the presence of lymphocytic infiltration, the typical nuclear inclusion bodies, and neuronal degenerative changes. Following corneal inoculation, the course of the virus along the ophthalmic division of the fifth nerve could be traced through the Gasserian ganglion into the brain stem. The virus then descended within the ventral portion of the spinal tract of V to the first cervical segment, occupying only that portion previously shown oscillographically to contain ophthalmic fibers.

The virus remained limited to the region occupied by the first division of V during the early stages of invasion only. Animals studied, whose death resulted from the virus, showed a diffuse invasion throughout the brain stem. The evidence indicates that the virus follows the primary neuronal processes initially infected to the region of their central telodendrons. Shortly afterwards, and from these regions, a rapid spread in many directions would seem to take place so that the usefulness of a virus in tracing neural pathways is limited to studies on the course of primary processes.

HENDERSON, J. W., see Arey et al.

HERTIG, et al. *On the seven and one-half day human ovum* (Carnegie no. 8020). Arthur T. Hertig and John Rock, Departments of Pathology, Obstetrics and Gynecology, Harvard Medical School and the Department of Embryology, Carnegie Institution of Washington.

The specimen was discovered after Bouin's fixation of a uterus removed surgically on the twenty-fourth day of a 27- to 31-day menstrual cycle. A single coitus was recorded 7½ days before operation. The blastocyst, now collapsed, appears to have implanted early in its seventh day of development on the posterior wall of the uterus, 13 mm. from the fundus and 7 mm. from the left side (corpus luteum on right). The ovum is superficially implanted but well attached at its embryonic pole, appearing as a shallow, saucer-like mass 0.46 × 0.42 mm. containing a 0.07 mm. embryo covered by the thin, transparent, abembryonic blastocyst wall.

The endometrium, microscopically in the twenty-second day of its cycle, is edematous with active secretion. The trophoblast at its maternal attachment has developed from two-thirds of the blastocyst wall. It varies in thickness from that of the unattached blastocyst wall to 0.083 mm. beneath the embryo. Its diameters vary from 0.30 to 0.45 mm. It is composed of two cell types, large pale polyhedral cells and enormous darkly staining masses of syncytium, the latter actively invading, surrounding and digesting endometrial stroma. The chorionic cavity measure 0.288 × 0.186 × 0.044 mm. The embryo, possessing already a plate of primitive entoderm, measures 0.126 × 0.092 × 0.044 mm. The amniotic cavity is a mere cleft twenty-four micra in diameter.

HERTIG, et al. *On the 9½-day human ovum* (Carnegie no. 8004). Arthur T. Hertig and John Rock, Departments of Pathology, Obstetrics and Gynecology, Harvard Medical School, and the Department of Embryology, Carnegie Institute of Washington.

The specimen was discovered, while fresh, in a uterus removed surgically on the twenty-fifth day of a 31- to 34-day menstrual cycle. The probable fertile coitus took place 9½ days previously. The ovum was implanted on the posterior wall near the cornual ostium corresponding to the recent corpus luteum. It appeared as a raised red spot 0.28 mm. in diameter, surrounded by a two-thirds semi-circle of brilliant hemorrhage or congestion 0.85 mm. in diameter (blood in trophoblastic lacunae). After fixation, the implantation site measured 1.0 × 0.69 mm. and was raised 0.26 mm. above the surface. Surmounting the elevation was a defect 0.36 × 0.21 mm., exposing the underlying ovum.

The endometrium, microscopically, is in the twenty-sixth day of its development. The ovum is two-thirds implanted, although its entire wall shows some trophoblastic development, most marked at the embryonic pole. The syncytiotrophoblast, its most conspicuous feature, possesses numerous lacunae, some containing maternal blood. The embryo, still a bilaminar germ disk, is developing an amniotic cavity 0.066 mm. of diameter from amnioblasts of trophoblastic origin. From the mid-section of the series the chorion measures 0.45 × 0.31 mm. It varies in thickness from 0.035 to 0.175 mm. The irregular chorionic cavity measures 0.185 × 0.12 mm. The embryo measures 0.10 mm. in diameter and 0.075 mm. in thickness, including the amnion and the entoderm.

HIGGINS. *The use of promin (sodium P.P' diamino-diphenyl sulfone–N.N' didextrose sulfonate) in nutrition experiments.* George M. Higgins, Division of Experimental Medicine, The Mayo Foundation.

Promin, shown to have therapeutic value in experimental tuberculosis, induces toxic changes in animals when given by mouth; but is relatively innocuous when given parenterally. Toxic changes include anorexia, loss in weight, alopecia, cyanosis, intravascular crenation of red cells, and a hypochromic anemia.

Young rats fed a basal ration consisting of sucrose, purified casein, salts and corn oil, and given thiamine, riboflavin, pyridoxine, calcium pantothenate, niacin and choline hydrochloride daily by mouth gained 22.7 grams per week. The oral administration of 50 mg. of promin daily to rats fed the same diet induced a weight loss of 1.7 grams at the end of the first week, a gain of 13.4 grams at the end of the second week and 6.8 grams at the end of the third. Rats fed the same diet and given the same amount of promin daily, but partaking ad lib of the vitamins provided separately in bottles, gained 11.5 grams during the first week, 16.8 grams during the second, and 14.5 grams during the third. The retarded growth, alopecia, the fall in red cell and hemoglobin levels were largely prevented by the increased intake of the vitamin fractions freely selected by the rats.

Promin may inhibit the intestinal synthesis of essential growth factors, as hitherto shown for sulfaguanidine (Black, McKibben and Elvehjem, 1942).

HIGGINS, et al. *The relation of vitamin B to the duration of anesthesia induced by pentobarbital sodium.* George M. Higgins and Frank C. Mann, Division of Experimental Medicine, The Mayo Foundation.

The duration of anesthesia, induced by pentobarbital sodium, has been studied in adult rats fed a basal synthetic diet, adequate except for vitamin B. Pentobarbital sodium was given intravenously in amounts equal to 25 mg. per kilogram of body weight. The basal diet consisted of glucose (69 parts), purified casein (20 parts), Crisco (3 parts), salt (4 parts), agar (2 parts) and cod liver oil

(2 parts). The vitamin B supplements, when provided, were given daily by stomach tube; each rat receiving 50 micrograms of thiamine, 25 micrograms of riboflavin, 25 micrograms of pyridoxine, 100 micrograms of pantothenic acid, 350 micrograms of niacin, 8 mg. of inositol and 5 mg. of choline.

Adult rats (258.9 gm.) feeding a commercial ration slept 28.8 minutes when anesthetized with pentobarbital sodium. Adult rats (244.0 gm.) feeding the synthetic diet supplemented by the tubing regime slept 26.6 minutes when anesthetized with the same relative amounts of the drug. When these same animals were fed the synthetic ration without the B fractions they slept 67.7 minutes when anesthetized with relative amounts of pentobarbital sodium. The prolonged anesthesia is not due to the restricted food intake of the vitamin B-free animals; but probably to the depleted tissue levels of the vitamin.

HOGUE. *Trichomonas vaginalis in tissue cultures.* M. J. Hogue, Department of Anatomy, University of Pennsylvania.

A bacteria-free culture of Trichomonas vaginalis was introduced into hanging drop tissue cultures of chick embryo intestine. The trichomonas swarmed along the cut edges of the embryonic tissues, and entered the lumen of pieces of the intestine. They swam freely over the fibroblasts, epithelial cells and sympathetic nerves. They attached themselves with their axostyles to the tissue culture cells and whirled about on them. They wandered about in a plexus of sympathetic nerves and rested on the nerves with their axostyles. Many individuals clumped together to form rosettes. In feeding over the tissue culture cells often they sent out a proboscis-like structure containing the flagella and undulating membrane. In the hanging drop they dragged and pushed around the plasma granules. They did not mechanically injure the tissue culture cells, though they gave off a substance which killed the tissue culture cells in 4–5 hours. Cultures of trichomonas heated at 56° for 15 and 30 minutes and at 60° for 20 minutes when added to the tissue cultures caused their death.

Metts tubes and Gillman and Cowgills test for proteolytic enzymes gave negative results.

HOOKER, C. W., see Atkinson et al.

HUBER. *Subdivision of the lung on the basis for bronchial distribution.* John Franklin Huber, Department of Anatomy, Temple University School of Medicine.

Each bronchus, from the largest to the smallest, supplies or spreads out to become a definitely delimited portion of the lung separate from the adjacent portions. These are natural subdivisions of the lung and can be readily demonstrated by inflation through individual bronchi or injection of colored gelatin into individual bronchi. The classical lobes of the lungs are portions of the lung each supplied by its own bronchus and are just as much entities whether separated by fissures or not. Here, then, we have a basis of subdividing the lung which allows us to continue the subdivision as far as desired, even down to the lung lobule of Miller. From the standpoint of the clinician it is now desirable to be able to talk about subdivisions smaller than the lobes.

It is suggested that the portion of the lung supplied by any bronchus can be called a ''bronchopulmonary segment'' and that we speak of first order bronchopulmonary segments subdivided into second order bronchopulmonary segments which in turn are subdivided into third order bronchopulmonary segments, etc. Each bronchial branch can then be designated as the bronchus to the ——— ——— bronchopulmonary segment, which would greatly simplify the naming of the bronchi.

HUMPHREY. *A lethal recessive character in the Mexican axolotl (Amblystoma mexicanum).* R. R. Humphrey, Department of Anatomy, University of Buffalo.

In many spawnings of axolotls of the partial albino strain, numerous embryos exhibit a striking defect in development which is apparently inherited as a simple Mendelian recessive. This abnormality consists in the accumulation of excess fluid, either in the archenteron, or between the neural tube and ectoderm of the head and neck. The condition becomes recognizable in embryos of early tail-bud stages as a distention of the head. Later, a marked lateral dilatation may develop dorsal to the branchial arches. Excess fluid in the archenteron occurs most often in embryos retarded in development by low temperature or other causes. The affected embryos may show much reduction of the dilated parts before hatching, but die subsequently from failure to begin feeding.

In thirteen spawnings in which this trait appeared, 1015 embryos were affected out of a total of 4163 living to the necessary stages. The percentage of abnormal embryos (24.4) thus approximates that expected when a trait depends upon a single recessive gene carried by each parent.

Embryos heterozygous for the character must develop normally, as a rule, since breeding tests show the gene to be carried by the majority of the adults of the present colony, animals retained chiefly because of superior size and vigor. The gene has been traced back to a female secured from The Wistar Institute colony in 1935.

HUNT. *Mitotic activity in the anterior hypophysis of rats during pregnancy and lactation.* Thomas E. Hunt, Department of Anatomy, University of Alabama.

Mitotic activity in the hypophysis of the rat is increased during the first three days of pregnancy. This period is about twice as long as that during which mitoses occur in the post-ovulatory phase of the estrous cycle. On the other days of pregnancy the mitotic activity is at a low level comparable to that found at diestrus. Animals killed on the first and second days after a litter is born again show an increased mitotic activity. During lactation this falls to a low, diestrous, level and is not increased until the post-ovulatory phase of the first cycle after weaning. There is some evidence that the post-parturient and post-lactational mitotic activity is greater than that found in the post-ovulatory phase of virgin rats of comparable age. Furthermore, since the period of mitotic activity at the beginning of pregnancy is prolonged, there is probably a greater increase in the total number of dividing cells than occurs during an estrous cycle. The fact that increased mitotic activity is found only after ovulation lends support to the idea that an ovarian hormone is a factor which induces cell division.

JANES. *Effect of chronic injections of diethylstilbestrol on the normal rat.* Ralph G. Janes, Department of Anatomy, Wayne University College of Medicine.

Five rats, two males and three females, received subcutaneous injections of 100 micrograms of diethylstilbestrol daily for 13 months. Determinations were then made for blood sugar, liver and muscle glycogen, and urinary nitrogen; various organs were weighed and studied microscopically.

The carbohydrate levels of the experimental animals were not raised above those of the controls. This is in contrast to previous observations that diethylstilbestrol, given for shorter periods, caused an elevation in liver glycogen. Urinary nitrogen values were variable.

The hypophysis was enlarged in all experimental animals, particularly in the males, each of which had a chromophobic adenoma of the anterior lobe. The adrenals weighed more in the injected animals, owing principally to an increase in the size of the zona fasciculata. Following appropriate staining, the liver of the treated rats showed slight abnormalities, namely, an increase in the size of the sinusoids and minor degenerative changes in some of the cells; liver glycogen when present, was evidenced as small granules near the central vein; lipoid when present was seen as small droplets near the hepatic trinity. Glycogen distribution in the controls was similar to that found in the treated rats, but more lipoid material was present in the liver of the controls. The testes and male accessory organs were severely damaged by the treatment and the ovaries were atrophic, the mammary glands showed evidence of stimulation. The thyroids and kidneys appeared unaffected.

JONES. *Mitotic activity of primitive erythroblasts increased by administration of antipernicious anemia preparations to pregnant rats.*[1] Oliver P. Jones, Department of Anatomy, University of Buffalo.

Additional experiments have substantiated previous claims (Jones, '39, '40) that embryonic erythropoiesis is influenced by the administration of antianemic substances to pregnant rats. The mean blood cell diameters of 96 embryos obtained from 51 pregnant rats, which had been treated with various liver extracts or ventriculin, were decreased so that 40–50% of cases fell below the normal 10 percentile of 13.8 microns. Control group consisted of blood from 54 embryos from 27 pregnant rats, sampled as described previously (Jones, '41, '42). In initial reports, it was considered that these results indicated accelerated maturation. To determine whether or not this was due to maturation, proliferation or a combination of these, mitotic phases per thousand cells were enumerated and karyological curves constructed. Some substance in antianemic preparations is transmitted across the placenta which stimulates cellular proliferation so that 75% of cells in late telophase fall above the 90 percentile of 11.4 per thousand. This accelerated proliferation increases the number of smaller daughter cells within the population thereby contributing to a reduction in mean cell diameters. Some maturation, as indicated by polychromatophilia, also accompanies this proliferation. When massive doses of iron are administered to pregnant rats, embryonic blood cells are also decreased in size. This is not due to proliferation but to accelerated maturation. Hence, a single cell type reacts differently to unrelated antianemic substances.

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[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

KEYES, J. T., see Carey et al. 1 and 2

KIRGIS, et al. *Regeneration of unmyelinated fibers following cervicothoracic and upper thoracic ganglionectomy.* H. D. Kirgis and E. A. Ohler, Department of Anatomy, Tulane University. (Introduced by Harold Cummins.)

To determine the extent of ascending regeneration of postganglionic fibers from thoracic ganglia of the sympathetic trunk, nineteen cats were subjected to ganglionectomy. The cervicothoracic and second thoracic ganglia were removed in seventeen cats; in two cats the third thoracic ganglion also was removed. Posterior root ganglia of the upper three thoracic segments were removed in two cats to obviate interpretation of unmyelinated sensory fibers as regenerated postganglionics. In five animals in which a cervicothoracic and second thoracic ganglionectomy was performed, a closely fitting glass or silver tube was applied to the cut end of the lower portion of the trunk. The upper end of the tube was sealed.

As early as three months postoperatively dissections demonstrated renewed connections between the sympathetic trunk and the upper thoracic and lower cervical nerves. The size of these bundles of regenerated fibers varied but in most cases they were relatively large. Preparations made by protargol and osmic acid technics showed many unmyelinated fibers. Great activity of the regenerative process was evidenced by the emergence of fibers from the lower end of the inserted tube from which point they took an ascending course.

Since the number of unmyelinated fibers was so large, and in view of their persistence following removal of posterior root ganglia, it was concluded that postganglionic fibers had regenerated from ganglia of the sympathetic trunk below the second and third thoracic.

KLEMME, R. M., see Minckler et al.

KNOUFF, et al. *The adrenal lipids of fasting and vitamin C deficient guinea pigs.* Ralph A. Knouff, Margaret C. Oleson, and Violet Wagner, Departments of Anatomy and Physiological Chemistry, Ohio State University.

The adrenals of one hundred and fifty animals have been examined. The right gland was used for chemical analysis, the left, for histological examination. Phospholipids, fatty acids, and total and ester cholesterol values were obtained for each gland by recently improved micro methods. The chemical data demonstrate a marked reduction in cholesterol esters in both the fasting and vitamin C deficient groups. Free cholesterol and phospholipid do not appear to be altered in either group. The fatty acids are slightly reduced in the starvation group but unchanged in the vitamin C deficient group.

Sudan Orange and the Schultz test for cholesterol were the histological methods employed. There was a marked reduction in Schultz-positive material in both experimental groups. In a majority of instances in both groups the sudanophil material was concentrated in the inner part of the cortex and greatly reduced in the outer portion. This material was usually Schultz-negative, rarely slightly positive. Moreover, in those cases in which chemical analysis revealed a high fatty acid content the inner sudanophil zone was unusually broad and dense. These features suggest that the inner fatty zone consists largely of neutral fats.

There is in general close correspondence between the amount of lipid revealed by histological staining and that determined by chemical methods. The hypertrophy of the adrenal was greater in the vitamin C than in the starvation group.

KORNHAUSER. *A quadruple tissue stain for strong color contrasts.* S. I. Kornhauser, Department of Anatomy, University of Louisville.

With the increasing use of kodachrome photomicrographs for revealing the finer structure of organs, it became advantageous to develop a staining combination to bring out in strong contrasting colors, the elements of tissues. This has now been accomplished using exclusively American made dyes, two of which, synthetic orcein and acid alizarine blue 2 B, have only recently been produced through the fortunate cooperation of the Commission on the Standardization of Biological Stains and two of our leading dye manufacturers. Zenker or Helly fixed material either paraffin or nitrocellulose embedded are stained on the slide first in a nitric acid alcoholic synthetic orcein solution which stains the elastic fibers only a dark red-brown. The second stain is an aqueous alum sulphate, ferric chloride, acid alizarine blue solution. Its purpose is to stain the nuclei and muscle fibers, and their color can be controlled from violet to purple by varying the amount of ferric chloride in the staining solution. Next the slides are put into a 5% aqueous phospho-tungstic acid solution. This decolorizes the collagenous fibers and cell cytoplasm and also mordants the sections for the third and final staining solution which is fast-green and orange G in 2% acetic acid. This stains collagen and basement membranes a brilliant green and erythrocytes, plasmasomes and myelin sheaths bright orange. The sections are dehydrated, cleared and mounted in xylol-clarite. Not only does our ''Quad'' stain bring out the ordinary tissue elements beautifully for observation and color photography but such obscure structures as basement membranes, neurofibrils and cell boundaries are clearly defined. Detailed directions will be published in Stain Technology.

KRAFKA, J., see Noback

KREDEL, F., see Rasmussen et al.

KUHLENBECK. *The derivatives of the thalamus ventralis in the human brain.* Hartwig Kuhlenbeck, Department of Anatomy, Woman's Medical College of Pennsylvania.

Nuclear configuration and fiber tracts of the human thalamus ventralis have been made the subject of an analysis based upon the previous comparative and embryologic studies of the author and his associates. In these studies the four longitudinal diencephalic zones and their derivatives were traced through the entire vertebrate series.

During mammalian ontogenetic development the primordium of the thalamus ventralis becomes separated from the thalamus dorsalis by the zone limitans intrathalamica in which the lamina medullaris externa and the fasciculus thalamicus (H_1) develop. A similar, but less conspicuous limiting zone separates the thalamus ventralis from the caudal part of the hypothalamus. This zone becomes the fasciculus lenticularis (H_2). The band of gray matter between these two fasciculi is represented by zona incerta and nucleus reticularis which form a continuous group of common origin. Both designations have been used vicariously in the wider sense for the entire complex.

The main nuclear groups distinguished in the human thalamus ventralis are: (1) the nucleus reticularis; (2) the zona incerta; (3) the nucleus filiformis (nucleus taeniae); (4) the ventral nucleus of the lateral geniculate body; (5) the ventral nucleus of the medial geniculate body (nucleus geniculatus medialis ventralis suprapeduncularis).

The subthalamic nucleus (of hypothalamic origin) and the nuclei of the fields of Forel (of hypothalamic or tegmental origin?) do not derive from the thalamus ventralis.

KUNTZ, A., see Harris.

LASSEK. *The pyramidal tract. A fiber and numerical analysis of the pyramid in the spider monkey (Ateleus Ater).* A. M. Lassek, Department of Anatomy, Medical College of the State of South Carolina.

The fiber components of the pyramid in the new world spider monkey have been studied because of its peculiar, digital movement. The hands of these animals have a well-developed palm, marked digits and complete absence of the thumb. Isolated, skilled movements of the hand are not possible with these characteristics. The feet have a long sole, small heel, a short opposable hallux and long toes. Their tail is long and is used dexterously as a prehensile organ, some authorities saying it merits the name of a fifth hand. It was found that the area and number of axons in the pyramids at a level above the decussation is very much the same as that in the old world macacus mulatta (rhesus) where skilled movements are better performed. On the basis of a preliminary study, the great majority of myelinated fibers measure between 1–4 μ, a few have diameters between 5–10 μ and a very small percentage have calibers between 11–12 μ. The largest myelinated fibers are about one-half the maximum found in the human. It is concluded from the study that the area and number of axons in the pyramids of some primates may not be proportional to the degree of specialized, digital activity.

LASSEK, A. M., see Schadewald

LATIMER, et al. *A case of hernia in the superior duodenal fossa.* Homer B. Latimer and Charles Wolfson, Department of Anatomy, University of Kansas.

Hernia in the superior duodenal fossa is sufficiently rare to warrant the recording of this additional case. The body was that of a well nourished Indian male; age, 36; a common laborer. The death certificate further states that death was due to coronary thrombosis.

The body, embalmed before shipment, was re-embalmed here with the usual alcohol-phenol-glycerine mixture intravenously, the cavities filled with 10% formalin, and tanked for about 7 months in a 2% phenol solution.

When the abdomen was opened in the course of the routine class dissection, the first fourteen inches of the jejunum were found enclosed in the superior duodenal fossa. This part of the intestine appeared perfectly normal, with only a very slight impression, one or two millimeters deep on one side only, made by the edge of the sac. The free, or anterior edge, of the distended fossa contained the inferior mesenteric vein and appeared tighter than the adjacent parts of this hernial sac. The opening faced inferomedially and was large enough to permit the easy withdrawal of the enclosed portion of the intestine and also permit of its being tucked back in the fossa.

We have no history of this case, but from the anatomical findings it would appear that this herniation caused the subject no discomfort and that a variable length of the jejunum may have been enclosed in this sac.

LEBLOND. *Behavior of acidophilic and basophilic colloid towards (radio-) iodine in resting and stimulated thyroids.* C. P. Leblond, Department of Anatomy, McGill University.

Observations on the thyroids by the "autograph method" were performed 15 hours after a single injection of 2 micrograms of radioactive iodine to three groups of rats (normal, hypophysectomized and treated with the pituitary extract Antuitrin-T).

In all the groups the radio-iodine was found to accumulate in the colloid, as previously reported for normal animals (Journal of Anatomy, in press). The reaction appeared very intense in the stimulated thyroid of pituitary-treated rats, in which most of the colloid was basophilic, and very faint in the resting gland of hypophysectomized animals, which contains mostly acidophilic follicles. However. in any given section the reaction produced by acidophilic colloid was greater than that elicited by basophilic; moreover, of the acidophilic colloid masses, the densest reacted most markedly, indicating the greatest accumulation of administered iodine.

Since follicles with dense acidophilic colloid were usually found in resting gland and, even when they were present in stimulated thyroid, were rather inactive as judged by cell height, it is unlikely that acidophilic follicles fix iodine more actively than basophilic ones. Therefore, the results are taken to indicate that the smaller amount of radio-iodine in basophilic follicles is due to a more rapid turnover and excretion.

LEWIS. *The role of the superficial gel layer in gastrulation of the zebra fish egg.*[1] Warren H. Lewis, The Wistar Institute.

Eggs are spherical because of uniform contractile tension of a superficial gel layer. Its contraction squeezes cytoplasm out of yolk to form blastodisc (abstract, Lewis and Roosen-Runge). Spherical yolk globules are squeezed into tetrakaidecahedrons and opaque yolk becomes translucent. Gastrulation. Contraction of yolk gel layer exerts continuous pull on edge of multicellular blastula disc and germ ring and at the same time thrusts yolk up against disc. The plastic disc is reduced to a thin sheet that slowly covers yolk as the latter continues to be pushed against it. As yolk gel layer contracts and decreases in area its inner surface solates to compensate. Solation is complete when blastopore closes. The blastula disc consists of rounded cells embedded in a semi-gel matrix covered externally by a cellular gel layer continuous with the yolk gel layer. It is separated from the yolk by a thin transparent fluid layer which acts as a hydrostatic cushion. The above is based on observations and numerous experiments. The latter also indicate that gel layers of yolk, blastodisc and cells are probably produced by reaction between cytoplasm and environment.

Gel layers and adhesion of cells to one another are probably essential for cleavage, blastulation, gastrulation, neural tube formation and many morphogenic processes in amphibia and other animals.

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[1] Aided by grants from the International Cancer Research Foundation and the American Philosophical Society.

LEWIS, et al. *The formation of the blastodisc in the egg of the zebra fish, Brachydanio rerio.*[1] Warren H. Lewis, The Wistar Institute, and Edward C. Roosen-Runge, Brown University.

The fertilized egg is nearly spherical. The animal pole soon flattens. An increase in the contractile tension of the superficial gel layer at the animal pole would account for this flattening. The blastodisc soon begins to appear over the flattened area. The continuous contractile tension of the superficial gel layer surrounding the yolk forces relatively clear cytoplasm through the flattened area to form the blastodisc. The flattened area acts as a sieve and holds back the yolk granules. After an apparent period of rest, the continued contraction of the superficial gel layer produces a rather sudden streaming of fluid cytoplasm and it is forced out of the yolk through the sieve to produce the rapidly enlarging blastodisc. The streaming subsides immediately after the first cleavage but reappears and subsides on a smaller and smaller scale with the next three divisions. The formation of the blastodisc is thus analogous to the formation of the hyaline caps of the amoeba and of the slime mold and is due to the same fundamental factors, namely, the contractile tension of a superficial gel layer which forces the more fluid endoplasm through a localized porous layer. (Illustrated with motion picture.)

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[1] Aided by a grant from the American Philosophical Society.

MACKLIN, C. C., see Hartroft

MACKLIN, C. C., see Macklin et al.

MACKLIN, et al. *Erythroblastosis fetalis in a macerated fetus, sib to a case of hydrops fetalis.* Madge Thurlow Macklin and Charles C. Macklin, University of Western Ontario Medical School.

In 1937 one of us (M. T. M.) reported before the American Association of Anatomists at Toronto a case of erythroblastosis fetalis in a case of hydrops fetalis. Believing that the condition of erythroblastosis was due to a dominant inherited factor, it was asserted that any subsequent pregnancies of this mother would be likely, in 50% of the cases, to result in a fetus with erythroblastosis. The mother gave birth to a macerated fetus 3 years later. This had so degenerated that excess hemopoiesis could not be diagnosed, but the liver debris was loaded with Prussian blue granules, indicating the presence of iron, and hence of blood destruction. The placenta showed marked fibrosis, and also some deposits of iron. Thus erythroblastosis fetalis was proved to exist in a macerated fetus born sib to a fetus with hydrops fetalis. Examination of the blood of the parents showed the mother to be Rh negative and the father Rh positive. This is the expected result in erythroblastosis. Of eight pregnancies, only two ended in living children.

MAGOUN, et al. *Conduction systems in the olfactory brain.* H. W. Magoun, W. A. McKinley and C. A. Fox, Institute of Neurology and Department of Anatomy, Northwestern University Medical School.

Information concerning olfactory conduction systems was gained by applying single shock stimuli to the cat's olfactory bulb and recording the evoked action potentials oscillographically from the exposed ventral surface of the telencephalon.

From the olfactory tubercle a negative potential was recorded with two peaks whose latencies following stimulation averaged 6 and 11 msec. With 2 stimuli, the neural elements responsible for the two peaks exhibited different recovery times and the first could be facilitated. Both peaks were eliminated by rostral section of the lateral olfactory tract.

From the pyriform lobe two negative potentials were recorded, the first large and the second small, with peaks about 8 and 20 msec. after stimulation. A second stimulus following the first abolished the later and facilitated the earlier potential. Cutting the lateral olfactory tract eliminated both these potentials.

A third and still later potential recorded from the pyriform lobe required stronger stimulation of the olfactory bulb and was not eliminated by cutting the lateral olfactory tract. It was lost after deeper section of the olfactory crus.

These results indicate the presence of a pathway running centrad from the olfactory bulb in the lateral olfactory tract and firing elements in the olfactory tubercle and pyriform lobe. They also indicate the presence of another pathway originating in the olfactory bulb and firing the pyriform lobe after lateral olfactory tract section.

MAGOUN, H. W., see Barnes

MANN, F. C., see Higgins et al.

MASON, K. E., see Wilson

MASSOPUST, L. C., see Carey et al. 1 and 2

McCULLOCH, W. S., see Bailey et al.

McKINLEY, W. A., see Magoun et al.

MENKIN. *Studies on the effect of the leukocytosis-promoting factor on the bone marrow.* Valy Menkin, Department of Pathology, Harvard University Medical School.

Earlier studies have shown that a pseudo-globulin can be recovered from various inflammatory exudates which, upon introduction into the vascular system of dogs, will induce a marked rise in the number of circulating leucocytes (Menkin, '40). Further studies on the purification of the material indicate that preliminary removal of the euglobulin and albumin fractions of exudate will yield an extremely potent leukocytosis-promoting factor in the pseudo-globulin fraction (Menkin, unpublished studies). This factor is absent in normal blood serum, but it is found in the blood serum of an animal having a concomitant inflammation (Menkin and Kadish, '42). This indicates that it reaches the bone marrow from the site of inflammation via the blood stream. Its effect is to induce a discharge into the circulation of immature leukocytes from the bone marrow. These facts have suggested the necessity of studying the effect of this substance on the hematopoietic tissue of the bone marrow. The femoral bone marrow of dogs was selected as material of study. In a few cases the marrow of ribs was likewise examined. In all cases it was found following even one injection of the leukocytosis-promoting factor that this material induces marked hyperplasia of the granulocytic elements of the bone marrow. The megakaryocytes are likewise increased in number. These facts definitely indicate that the leukocytosis-promoting factor not only induces a discharge of leukocytes from the bone marrow, but it also stimulates active growth of some of the hematopoietic elements. This substance therefore offers a reasonable explanation for the mechanism of leukocytosis with inflammation by causing active hyperplasia of the bone marrow as well as discharge of immature leukocytes into the circulating blood.

METTLER. *Reflexes and recovery of motor capacity in the monkey after extensive chronic unilateral cerebral removals.* Fred A. Mettler, Department of Neurology, College of Physicians and Surgeons, Columbia University.

Comparison of monkeys from which all cerebral cortex of one hemisphere had been removed with those from which the corpus striatum had also been removed disclosed that latter showed greater patellar reflex amplitude of brisker quality than former. In both cases the plantar reflexes were sluggishly flexor. Both groups of animals were ultimately able to use extremities of both sides in walking, climbing and feeding but latter animals never used those of contralateral side alone. The former animals showed no degeneration of substantia nigra, the latter showed extensive but incomplete degeneration. Following hemidecortication both caudate and putamen were reduced in size, but cells of both types persisted and were more closely placed than usual.

Stiffness and contractures of the limbs were not seen until the removals were enlarged to include subthalamus. Under such circumstances the patellar reflex threshold, amplitude and force were reduced and no plantar response could be elicited. The contralateral limbs displayed sustained resistance to passive movement and, ultimately, contractures.

In summary: unilateral removal of the corpus striatum and cortex produces a greater alteration in the patellar reflex than is found after complete decortication of the hemisphere. It is not followed by any appreciable degree of resistance to passive movement nor by contractures. The contralateral limbs are freely employed in associated movements but are scarcely ever employed except in conjunction with the opposite extremities.

MICHELS. *The variational anatomy of the hepatic and cystic arteries.*[1] N. A. Michels, Daniel Baugh Institute of Anatomy, Jefferson Medical College.

The arterial blood-supply of the liver is so markedly varied that (as in the case of the spleen) no two patterns are the same. In fifty dissections of the celiac axis and its branches, the hepatic showed variations in the number, caliber, origin and distribution of its main branches (2–5), the small terminal branches (10–30) being prevailingly distributed to the caudate and quadrate lobes. Accessory hepatics occur frequently, complicating thereby not only the patterns of the blood-supply to the liver, but also those of the gallbladder, stomach, and diaphragm.

The cystic is extremely varied in length, caliber, sites of origin (high or low) and in its relation to the hepatic duct, the latter being prevailingly ventral to the right hepatic. Frequently double, the cystic may in instances be mistaken for a branch (inferior lobular) of the hepatic, selectively distributed to a notched area. Anastomoses comprise those between right hepatic and left hepatic, between left hepatic and accessory left hepatic, between left hepatic and esophageal branch of the left gastric. A constant first branch of the gastroduodenal is the supraduodenal artery distributed not only to the anterior and posterior walls of the first part of the duodenum (Wilkie) but frequently to the entire posterior surface of the duodenum. It is not an end artery, but shows many anastomoses.

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[1] Aided by a grant from the American Philosophical Society.

MINCKLER, et al. *The premotor cortical connections in man.* Jeff Minckler, Roland M. Klemme, Dona Minckler, Department of Anatomy and Section of Neuro-Surgery, St. Louis University School of Medicine.

Weigert and Swank-Davenport preparations of the brain from a patient subjected to bilateral premotor cortical excision reveal two distinct efferent bundles from areas 6 and 8: (1) commissural, passing through the posterior one-half of the body of the corpus callosum to the premotor cortex of the opposite side; (2) projectional, passing through (a) the posterior limb of the internal capsule near the genu, (b) the lateral one-half of the medial third of the cerebral peduncle in the midbrain, (c) the medial aspect of the corticospinal field in the pons, (d) diffusely through the pyramid in the medulla, and (e) through the medial aspect of the ipsilateral anterior white column of the lower medulla into the spinal cord. Neither long nor short association bundles efferent from the premotor cortex could be identified. Evidence suggests terminal relationships of the premotor bundle to (a) thalamus, (b) substantia nigra, (c) stem nuclei, (d) anterior horn of opposite side.

MINCKLER, J., see Saccomanno

NOBACK. *The placentation of a guinea baboon (Papio papio).* Charles R. Noback, Department of Microscopic Anatomy, University of Georgia, School of Medicine. (Introduced by Joseph Krafka.)

This abstract is a brief description of the placentation of a guinea baboon (Papio papio) obtained from the Zoological Society of San Diego. The external anatomy of the embryo, which was 5.2 mm. long (formalin fixed), was at a developmental stage similar to that of the 27-day-old macaque embryo. The discoid chorio-allantoic placenta of the hemochorial morphological type was implanted in an antimesometrial location on the ventral wall of the body of the uterus. There was no secondary or accessory placenta. The borders of the placenta were undercut by the uterine cavity with a fold of maternal endometrium at the free margin of the placenta. There was no decidua capsularis. Approximately one-fourth of the chorion was membranous chorion located abembryonically. The other three-fourths of the chorion was placental chorion. The umbilical cord was attached near the center of the placental disc.

OLESON, M. C., see Knouff et al.

OHLER, E. A., see Kirgis et al.

PAGE, et al. *The response of vessels of the mesentery to injections of renin and angiotonin.* Irvine H. Page and Richard G. Abell, Lilly Laboratory for Clinical Research, Indianapolis City Hospital, and Department of Anatomy, University of Pennsylvania Medical School.

It has recently been shown (Abell and Page, J. Exp. Med., '42, vol. 75, p. 673) that in hypertension the arterioles in transparent moat chambers in rabbits' ears become chronically constricted. Such constriction is not severe enough to stop the blood flow, but is great enough to increase the peripheral resistance to such flow. Similar, but temporary, constriction of the arterioles occurs in normotensive rabbits following intravenous injections of renin and angiotonin (Abell and Page, J. Exp. Med., 1942, vol. 75, p. 305). These findings are in accord with work by Page and his associates which suggests that hypertension may be caused by a renin-angiotonin vasopressor mechanism.

The present experiments were undertaken to test the effects of renin and angiotonin upon the arteries in the splanchnic area. Intestinal-mesenteric chambers (Zintel, Anat. Rec., '36, vol. 66, p. 437) were installed in rabbits anaesthetized with pentobarbital. Between 2 and 6 days later, after the effects of the anaesthetic had entirely worn off, intravenous injections of renin (0.2 to 0.6 cc.) and of angiotin (0.5 to 1.0 cc.) were made. The reactions of the vessels in the portions of the mesenteries exteriorized in the chambers were observed with the microscope, and photographed. The arteries of the mesenteries constricted, just as those of the ear. This suggests that in hypertension arterial constriction in the splanchnic region may play a part in the increased peripheral resistance to blood flow that is known to occur in this condition.

PALAY. *The origin of neurosecretory granules from the nuclei of nerve-cells in fishes.* Sanford L. Palay, Department of Anatomy, School of Medicine, Western Reserve University. (Introduced by Ernst A. Scharrer.)

In the course of a study of the role of cellular constituents in the elaboration of neurosecretory granules, nuclear secretion was found in the diencephalon of the catfishes Noturus flavus and Ameiurus nebulosus. The nuclei of the nerve cells in the nucleus lateralis tuberis are often subdivided into lobes interconnected by tenuous bridges. The nuclei also show deep invaginations filled with basophil cytoplasm. The nucleoli are single and large or multiple and small. The secretory granules lying within these nuclei are highly refringent, spherical, acidophil bodies of varying size. The whole nucleus or one of its lobes may be converted into a vesicle containing acidophil granules. These are extruded from the nuclei directly into the cytoplasm or into nuclear invaginations. Granules also appear in the Nissl substance. Occasional cells have granules not only in the nucleus, but also in the basophil cytoplasm of the nuclear invaginations and in the Nissl substance. Similar nuclear inclusions occur in the nucleus preopticus, although there the cytoplasmic type of neurosecretion prevails. According to these observations which agree with findings in certain other species of fishes, the basophil substances, i.e., the chromatin of the nucleus and the Nissl bodies of the cytoplasm, are the antecedents of neurosecretory material, whereas nucleoli, mitochondria, and Golgi apparatus do not visibly enter into these processes.

PEARSON. *Sensory type neurons in the hypoglossal nerve.* Anthony A. Pearson, Loyola University School of Medicine.

Bipolar neurons were found in the hypoglossal nerve of human fetuses of four, five and six months of age. These neurons were spindle shaped nerve cells with a central and a peripheral process. They were located in the intramedullary course of the hypoglossal nerve in the region of the hilus of the inferior olivary nucleus. The character of these neurons suggests that they are sensory in function.

PEARSON, O. P., see Enders et al.

PIATT. *The course and decussation of ectopic Mauthner's fibers in Amblystoma punctatum.* Jean Piatt, Department of Anatomy, University of Pennsylvania.

Supernumerary Mauthner's cells were produced in Amblystoma punctatum larvae by grafting homoplastically the embryonic medulla and its associated structures in place of the forebrain. Fifteen bilateral and fourteen unilateral transplantations were made.

No striking uniformity was observed in any of the following: (1) Decussation across median plane of brain and corresponding site of occurrence; (2) Dorsal, as opposed to ventral, decussation; (3) Impulsion of decussation due to presence of contralateral cell; (4) Initial direction of growth, i.e., anteriorly or posteriorly; (5) Association of axon with a particular tract or tracts throughout its course in either brain or cord.

Uniformity was observed, with some exceptions, in the following: (1) Mid-line decussation occurred far anteriorly to that of the normally placed host cells; (2) Decussation of axons themselves and the inter se crossing of their respective branches occurred with left fiber dorsal to right; (3) Decussation occurred at a standard or normally located decussation or commissure; (4) Predilection for a route in the ventral or motor portion of cord.

These and numerous additional data suggest that the accessory fibers, and presumably the normal fibers of Mauthner also, attain their definitive anatomical relationships by reason of their ductility to the over-all plan of growth of the nervous system, and not through a dependency on preexisting structures as such.

PICK, J. W., see Anson et al.

RAMSAY. *The drainage pathways of lymph from the thyroid gland (cat).* A. J. Ramsay, The Daniel Baugh Institute of Anatomy, Jefferson Medical College.

This paper is accompanied by a motion picture which illustrates the lymph drainage of the thyroid gland.

Diluted carbon ink, injected under the capsule of one lobe, is seen to inject the entire gland by quickly spreading across the area of the isthmus to the opposite side. Although no parenchymatous isthmus is present in the adult cat the lymphatic space which surrounds the fetal isthmus is retained, serving as a lymphatic connection between the two thyroid lobes.

Immediately following injection of the gland proper, the ink enters lymphatic vessels which drain the extensive thyroid lymphatic spaces. The principal drainage pathways are: Superiorly to deep cervical nodes; inferiorly to tracheal, jugular, suprasternal and superior mediastinal nodes; directly to the internal-external jugular vein junction, directly to the jugular-subclavian vein junction, or, at times, directly to the cervical lymphatic trunks themselves.

RAMSAY, et al. *Histological changes in pancreatic acini in response to peptone solution in the small intestine.* A. J. Ramsay, J. E. Thomas and J. O. Crider, Departments of Anatomy and Physiology, Jefferson Medical College.

This study reports the histological changes seen in pancreatic acinar cells when stimulated by the presence of peptone in the intestine. Three groups of experiments were performed; the results are as follows:

Group I. Pancreas samples from dogs with intact vagus nerves show secretory stimulation at the end of the first hour of peptone administration. Later samples show still more marked loss of zymogen granules and accumulation of stainable secretion in the smaller ducts. This histological picture is comparable to that following electrical stimulation of the vagi.

Group II. Vagotomized (bilateral) dogs show no histological evidence of secretion.

Group III. Dogs with vagi intact but with stomach and duodenum separated (to prevent gastric secretions from reaching the duodenum) show secretory stimulation similar to, although not so marked as, the average response seen in Group I.

These results show that peptone, in the intestine, stimulates the pancreas to secrete in a manner similar to that following electrical stimulation of the vagi. Further, the mechanism through which products of protein digestion stimulate pancreatic secretion is again shown to depend upon the integrity of the vagus nerves.

RASMUSSEN, et al. *Morphological changes in the sciatic nerve of dogs following nerve traction.* G. L. Rasmussen and F. Kredel, Departments of Anatomy and Surgery, Medical College of the State of South Carolina. (Introduced by George Clark.)

Traction of forty-one dog sciatic nerves was accomplished by means of a glass hook 9 mm. in diameter to which was attached a weight by a connecting string that passed over a pulley. Weights of 300, 500, 1000 or 1500 grams lifted the nerve at right angles. They were applied for durations of 5, 10, 15, 20 and 30 minutes. The animals were sacrificed at intervals of 10 minutes to 27 days. Teased and interrupted serial sections of each nerve were studied by the Marchi, silver and Masson technics. The duration of traction was found to be an important factor. For example, weights of one kilogram or less produced only myelin and Schwann sheath alterations at the site of the hook and distal to it when allowed to remain in 5–10 minutes. Comparable weights left for 15–30 minutes showed more marked periaxial alterations plus degenerative changes in the endoneurium and nutrient vessels. In contrast, a 1500 gram pull for 5 minutes was sufficient to cause fracturing of the axons resulting in typical Wallerian degeneration. Although the strain was practically equal on either side of the hook the central portion remained normal in all experiments. This fact coupled with certain vascular changes suggests that circulation may have been sufficiently impaired distally to account for the periaxial changes.

RATHBUN, E. N., see Brues et al.

REED, H. W., see Bennett et al.

RHINES. *Development of motor nuclei and fiber tracts after removal of large portions of the brain.* Ruth Rhines, Department of Anatomy, Northwestern University Medical School. (Introduced by W. F. Windle.)

Bok ('15) proposed that growth of axons of somatic motor neuroblasts in the brain is initiated by arrival of caudally growing diencephalic fibers of the medial longitudinal fasciculus. Thus motor nuclei differentiate in cephalo-caudal sequence.

Chick embryos in which the diencephalic medial longitudinal fasciculus was prevented from forming by removal of the forebrain, including the primordium of the interstitial nucleus, had well-developed motor nuclei. In 4-day embryos, the oculomotor nucleus and nerve were differentiated in the absence of any longitudinal fibers; the tröchlear nucleus was normally developed, although no descending and only five ascending fibers were present at that level. Vestibular components of the medial longitudinal fasciculus do not activate motor nuclei in the absence of the diencephalic component, for ascending fibers do not reach the trigeminal region until after 5 days. The abducens nucleus, appearing at 60 hours, precedes development of descending vestibular fibers which are not apparent until after 4 days. Fiber tracts of operated embryos develop in their normal pattern despite loss of large amounts of the brain. With eyes and forebrain absent, tectum and tectal tracts differentiate normally. After removal of the brain rostral to the trigeminal region, tracts ascend and even emerge from the end of the brain. These experiments lend no support to the theory of stimulogenous fibrillation (Bok).

ROCK, J., see Hertig et al. 1 and 2

ROOSEN-RUNGE, see Lewis et al.

ROSEMAN, E., see Bailey et al.

RUGH, R., see Saunders et al.

SACCOMANNO. *Notes on the structure and innervation of the skin of the porcupine (Erethizon).* Geno Saccomanno, Department of Microanatomy, St. Louis University School of Medicine. (Introduced by Jeff Minckler.)

The epidermal strata are poorly defined. The papillary and reticular layers of the dermis are indistinguishable. The quills arise in follicles comparable to hair follicles. As the quill approaches maturity the follicle undergoes partial degeneration, which usually involves complete atrophy of the papilla and leaves the quill loosely attached. Sweat-glands are present only in the paw pads, where they are abundant and large. The sebaceous glands vary in size from structures comprising a few cells to bodies over 1 cm. in diameter. The ducts of several sebaceous glands may open into a single quill follicle. The arrector pili muscles also vary in size. The larger ones manipulate several quill follicles which are interconnected at their bases by compact connective tissue. The follicles of the guard hairs on the snout and those of other hairs and quills are surrounded by abundant plexiform networks of nerve-fibers. Lamellated end-organs also occur in the dermal papillae in the paw pads. Nerve-fibers appear to be intimately associated with sweat-glands, sebaceous glands, arrector pili muscles and cutaneous blood-vessels. Terminal branches also appear in the deep layers of the epidermis.

SAUNDERS, et al. *The x-radiation of eggs of Rana pipiens at various maturation stages.* Grace Saunders and Roberts Rugh, Department of Biology, Washington Square College of Arts & Sciences, New York University.

This study was aimed at an analysis of the differences of frogs' eggs and sperm (Rugh, '39) measured by their respective resistance to x-radiation as demonstrated in embryonic development. For the irradiation of ovarian eggs, which are in the germinal vesicle stage; and uterine eggs, which are in the metaphase of the second maturation division, the female donors were irradiated in the region of the ovary and uterus respectively. In doing this a marked difference in susceptibility of the eggs of these two groups was noted. At dosage levels of 1000 roentgen units, the percentage of normal tadpoles developing from treated ovarian eggs was approximately nine times that of irradiated uterine eggs. The work thus far has also been found to contrast with that done on sperm in that 58% of the ovarian eggs treated with 10,000 r. hatched, while only 2.4% of those fertilized by sperm treated with a similar dose was found to hatch. This result may be due to the difference in the stage of nuclear maturation of these eggs and sperm and to the differences in the amount of yolk and cytoplasm. At all irradiation levels the eggs seemed to be more resistant to damage than did the sperm of the previous investigation. Further work is being done on eggs from the body cavity, which are in the first maturation division; eggs immediately after insemination and prior to the giving off of the second polar body; and eggs immediately after the second polar body has been given off.

SCHADEWALD. *The absence of arterio-venous anastomoses in human stellate ganglia.* Melvin Schadewald, Department of Anatomy, Medical College of the State of South Carolina. (Introduced by A. M. Lassek.)

Nonidez ('42) has described in detail arterio-venous anastomoses present in the stellate and upper thoracic chain ganglia of dogs. A careful study of 37 right human stellate ganglia revealed the presence of no comparable structures in man. This finding is in agreement with the reported absence of such structures in the thoracic sympathetic of a rhesus monkey (Nonidez, '42) and adds emphasis to the probability of species differences in the structure of these ganglia. The majority of the cases used in this study were sectioned serially after formalin fixation and stained with hematoxylin and eosin, thionin or cresyl violet, Mallory-Heidenhain trichrome, and activated protargol. The ages of the individuals from whom the ganglia were removed at autopsy ranged from new-born to 85 years. The cases were carefully analyzed and considered in the eventuality of possible involvement of these ganglia in pathologic processes; hence, this factor has been obviated. A complete account of the cases studied is available in a thesis presented by the author to the graduate school of the University of Minnesota in June, 1942.

SCHAEFFER, J. P., see Bates

SCHARRER. *Hemopoiesis in the meninges of ganoid fishes.* Ernst A. Scharrer, Department of Anatomy, School of Medicine, Western Reserve University.

The large mass of tissue over the fourth ventricle of the ganoid brain (Tilney's ''metaphysis'') has been studied in Amia and Lepisosteus. The specimens used were fixed by vascular perfusion immediately after they were caught in Lake Erie. This tissue derives from the meninges and in Amia approximately equals the brain in volume. A typically myeloid tissue, it possesses a stroma of argyrophil fibers which are connected with the blood-vessels. The sinusoids are lined by littoral cells which store trypan blue. These may round off as free macrophages. No other cells are seen to store trypan blue, and primitive reticular cells have not been identified. In Amia fat cells surround this myeloid tissue but are not interspersed through it. Large melanophores are numerous. A rich innervation can be demonstrated. The free cells include stages in the development of lymphocytes, granulocytes, and erythrocytes. The erythrocytes can be observed on their way into the sinusoids. They show narrow constrictions of nucleus and cytoplasm where they pass through openings in the walls of the sinusoids. This meningeal myeloid tissue also fills cavities in the cartilaginous skull that are lined with thin layers of bone. It thus fulfills the requirements of true hemopoietic bone marrow which here makes its first appearance in the vertebrates.

SCHARRER. *The role of the Nissl bodies in neurosecretion in Amphibia.* Ernst A. Scharrer, Department of Anatomy, School of Medicine, Western Reserve University.

Palay[1] demonstrated in fishes the origin of neurosecretory granules from nuclei and Nissl bodies. The observation of nuclear secretion appears unique; in other vertebrates the nucleus has not been found to participate so conspicuously in the elaboration of neurosecretory granules. The demonstration of Nissl bodies as another source of secretory material may be applied, however, more generally. Here observations in Amphibia are reported. In the nucleus preopticus of the toad the smallest granules occur among the Nissl bodies which lie in the periphery of the cell body. As soon as the granules become larger they replace the Nissl substance, which disappears during the elaboration of the granules. Only when the granules coalesce to form larger droplets, do they fill the cell body without relation to the Nissl zone. Since the Nissl bodies have been shown by the freezing-drying technique to be segregated in groups (Bensley and Gersh, '33), this spatial relationship between the Nissl bodies and the neurosecretory granules should be significant and should have an equivalent in the living cell. To consider the Nissl substance as a precursor of the secretory material in gland-like nerve cells is consistent with the role attributed to the basophil substances as antecedents of the secretory granules in other gland cells.

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[1] See Abstract p. 43.

SCHARRER, E. A., see Palay

SCHMITZ. *The substantia nigra and the entopeduncular nucleus in the cat.* John T. Schmitz, Department of Anatomy, Marquette University School of Medicine. (Introduced by Clement A. Fox.)

While investigating the possibility of retrograde cell degeneration in the amygdala, lesions were produced in kitten brains (Horsley-Clarke technique) medial to this complex. Results, other than those expected, appear interesting in the light of recent reports on the substantia nigra and the globus pallidus.

A lesion medial to the entopeduncular nucleus and extending into the fields of Forel interrupted the pallidofugal fibers in one brain. The entopeduncular nucleus degenerated completely while the globus pallidus remained intact. This result suggests the homology of the entopeduncular nucleus with the medial segment of the primate globus pallidus.

The cells of the substantia nigra, including those of the so-called "nucleus of the mammillary peduncle," disappeared in four brains with lesions in the ventromedial end of the internal capsule, destroying the entopeduncular nucleus. These results indicate the cephalic direction of the axones of the substantia nigra and agree with the observations of Keller and Harris ('41) and the Marchi experiments of Ranson and Ranson ('41) and Kimmel ('42).

The subthalamic nucleus, part of the mammillary body and the hypothalamus were injured in two of the above four brains, but none of these nuclei was involved in the remaining two brains. Since the nucleus of the mammillary peduncle degenerated equally in all four brains, it seems likely that this nucleus does not contribute fibers to the mammillary peduncle.

SCHMITZ, J. T., see Carey et al. 1 and 2

SCHOEMAKER, D. M., see Cross

SCOTT, J., see Ward et al.

SELYE. *Nephrosclerosis and tissue edema after desoxycorticosterone treatment.* Hans Selye, Department of Anatomy, McGill University.

It has been shown (Selye, Canad. Med. Assoc. J., vol. 47, pl. 515, '42) that overdosage with desoxycorticosterone acetate (D.C.A.) causes nephrosclerosis, tissue edema, ascites and cardiac hypertrophy in the chick. All these changes are much more readily produced if the chicks are given 0.3% NaCl solutions instead of drinking water. Without D.C.A. such dilute saline solutions cause no distinct pathological changes. However, 1 to 2% NaCl imitates D.C.A. overdosage in every respect. Hence the toxicity of D.C.A. may primarily be due to its ability to sensitize the body to NaCl.

In humans D.C.A. also causes edema. We were surprised, therefore, to find that in the dog, or rhesus monkey, doses of 40 mg. and in the rat 10 mg. of D.C.A. per day given for several months elicited no tissue edema even if given in combination with 1% NaCl instead of drinking water. However, spells of paralysis may be elicited at will in dogs and monkeys by NaCl administration.

SELYE, H., see Clarke et al.

SENSENIG. *The development of the caudal vertebrae in the deer-mouse (Peromyscus maniculatus rufinus).* E. Carl Sensenig, University of Alabama School of Medicine. (Introduced by Charles M. Goss.)

The migration and distribution of sclerotomic tissue and its future development into vertebrae was traced in the caudal region of the deer-mouse. These observations were then compared to those of a typical thoracic segment. The following variations occur in the caudal region:

1. Cell migration is retarded by the disproportionately large size of the neural tube.
2. Cell density is uniform throughout all parts of the sclerotomites with a greater density in the caudal sclerotomite than in the cranial sclerotomite.
3. The sclerotomites share equally in the formation of the elongate centra with the caudal sclerotomite predominating in the formation of the reduced intervertebral discs.
4. Dorsal and ventral sclerotomic migrations occur only at the anterior and posterior ends of the sclerotomes forming rudimentary neural and haemal arches. These in the definitive vertebrae appear as paired dorsal and ventral bosses which serve in the articulation of the vertebrae.
5. Chondrification first appears in the mid-portion of the membranous centra surrounding the notochord. From here it spreads throughout the centra and into the arches which have no chondrification center of their own.

SILVEIRA, A., see Bailey et al.

SIMER. *The drainage of particulate matter from the peritoneal cavity by omental lymphatics.* Parke H. Simer, Department of Anatomy, University of Illinois, College of Medicine.

Diluted india ink was injected into the peritoneal cavities of nineteen cats. The animals were killed at periods varying from 1 hour to 1½ years after injection and their thoracic and abdominal lymphatics studied grossly and microscopically. In the gross specimen the position of each sternal lymph trunk was always indicated by a dark line, but microscopic study showed an interesting contrast. In animals injected as recently as 1 hour to 3 days, the trunks were filled with free ink particles and large, overladen phagocytes, but in those animals injected weeks or months previously, the trunks were empty and the dark line was due to an accumulation of ink-filled phagocytes adjacent to the lymph vessel.

Since most of the omental lymph vessels drain to the duodenal node, near the pylorus, the degree of coloration of this node was considered a fair index of the functional activity of the vessels. Ink was first observed in the node at its caudal end, twelve hours after injection. The coloring showed no marked increase, either in amount or distribution, in those animals injected for longer periods. Microscopic sections of omental fat strips showed a few phagocytes in the larger lymph vessels, but none in those associated with the central artery.

It is concluded from this study that omental lymphatics are of little importance in removing particulate matter from the peritoneal cavity.

SIMKINS. *Functional anatomy of the eustachian tube.* Cleveland S. Simkins, Department of Anatomy, Creighton University Medical School.

The lumen is a semitubular slit curved in the shape of a gouge except at the pharyngeal ostium and the isthmus. Its walls are always in contact. The cartilage is hooked into the lateral tubal tissue like a continuous hinge and swings medio-inferiorly downward over the lumen in order to insure uniform adjustment of the walls for the passage of air in pressure equalizations. Two muscles, the salpingopalatinus and salpingopharyngeus straddle the levator which forms a roll along the bottom of the ostium against which they exert tension thus keeping the ostium closed as long as the soft palate is pulled downward and forward as in normal breathing. When the soft palate is elevated and tensed in the act of swallowing, tension upon the flutter valve is relaxed, and the mucous blanket is torn into a slit-like opening which endures for one or two-fifths of a second, enabling air to pass from higher to lower pressures. When the act of swallowing is over the torn blanket reunites and the soft palate swings forward closing the tube again. The mucous blanket being constantly swept toward the throat by the cilia cannot normally act as a plug to the ostium.

The tube is not actively opened, but it is actively closed. It is opened by air pressure and that has a distinct individual variation in value.

SNYDER. *The failure of expansion of fetal pulmonary alveoli associated with complete obstruction of the upper air passages.* Franklin F. Snyder, Department of Anatomy and Department of Obstetrics, Harvard University.

Through the fortunate chance of the spontaneous birth at term of a rabbit fetus in which the entire head region was formed so defectively that there was no external opening of the air passages, it was possible to observe the degree of expansion of the pulmonary alveoli when obstruction of the entrance of amniotic fluid into the lungs was complete. The influence upon the structural development of the lungs related to the tidal flow of amniotic fluid in the respiratory tract before birth could be evaluated from a new standpoint, namely, the complete absence of this exchange at full term.

Histological examination revealed extreme failure of expansion of the alveoli throughout both lungs. The large bronchi were patent while inconspicuous clefts marked the site of unexpanded alveoli. These findings were in striking contrast to those in normal control animals which had been sacrificed at term before breathing air. In controls definite dilatation of the alveoli was regularly found.

SOLNITZKY, et al. *The lateral geniculate complex of Ateles ater.* Othmar Solnitzky and Pinckney J. Harman, Georgetown University Brain Research Institute.

The lateral geniculate complex of the spider monkey, Ateles ater, consists of three distinct nuclear masses: the nucleus geniculatus lateralis (pars dorsalis), the nucleus pregeniculatus and the nucleus intergeniculatus.

The optic tract as it approaches this nuclear complex breaks up into seven major fascicles. The lateral six fascicles end in the nucleus geniculatus lateralis. The most medial fascicle splits into a lateral and medial subdivision; the former penetrates the medial part of the nucleus pregeniculatus, the latter curves around the peduncle to become lost in the fiber complex of the subthalamus.

The nucleus geniculatus lateralis (pars dorsalis) is distinctly laminated especially in its caudal part and subdivided into two superficial magnocellular and four deep parvocellular laminae. The medullary laminae, with the exception of the first, are very narrow. The hilus is directed ventromedially.

The nucleus pregeniculatus is subdivided into a medial and a lateral part; the lateral portion is in intimate relation to the reticular nucleus and the medial to the zona incerta and the optic tract.

The nucleus intergeniculatus (pars inferior of the pulvinar) separates the lateral and medial geniculate nuclei and shows extensive cellular fusion with both. It expands ventrolaterally and eventually displaces the nucleus geniculatus lateralis as the latter dwindles caudally. The nucleus intergeniculatus may be subdivided into a dorsomedial and a ventrolateral portion on the basis of cyto- and myeloarchitecture.

SOLNITZKY, O., see Harman et al.

STOWELL. *A spectrophotometric analysis of the staining of tissues.* R. E. Stowell and V. M. Albers, Barnard Free Skin and Cancer Hospital and C. F. Kettering Foundation for the Study of Chlorophyll and Photosynthesis, Antioch College.

In the production of skin tumors in mice by methylcholanthrene, it was important to determine the absorption spectra of stained tissues for the photometric histochemical estimation of variations in specifically stained tissue elements. (Stowell, J. Nat. Cancer Inst., v. 3, '42.)

By comparing spectral absorption curves of staining solutions and of substances stained with these solutions, information is obtained regarding the chemical changes associated with tissue staining. If the absorption spectra of the staining solutions and stained tissues are the same, no appreciable change in the chromophore groups has occurred as a result of the staining. The stains measured are acid fuchsin, aniline blue, azocarmine G, basic fuchsin, eosin Y, orange G, picric acid, and Sudan IV. The substances stained are gelatin, tendon, blood plasma, thymus gland, and fat.

Aqueous basic fuchsin and fuchsin-sulfurous reagent to which formaldehyde was added (Schiff reaction) represent definitely different dyes. The curves for thymus stained by the Feulgen technique and of the products of the Schiff reaction are similar.

The spectral absorption curves for staining solutions and substances stained with these solutions are comparable. With the spectrophotometric methods and stains employed, there is no evidence of significant chemical change in the chromophore radicals of the stains associated with the process of tissue staining. The results are compatible with the physical theories of staining.

THOMAS, J. E., see Ramsay et al.

TOBIN. *Effect of ovary and adrenal on the absorption of subcutaneous sesame oil in rats.* Charles E. Tobin, Department of Anatomy, The University of Rochester, School of Medicine and Dentistry.

Previous studies have shown that sesame oil is not bland when used for control injections or as a vehicle for fat-soluble hormones. Experiments reported here demonstrate further that removal of the ovaries or the adrenals, or both, definitely influences the absorption of sesame oil injected subcutaneously in amounts of 1 cc. at the time of operation and recovered 12 to 14 days later as an ether-soluble extract of the subcutaneous injection site. Average determinations based upon groups of five to twenty rats indicated that normal males utilized 51.8%; castrate males, 52.3%; normal females, 52.3%; spayed females, 31.4%; adrenalectomized males, 49.3%; adrenalectomized females, 42.2%; adrenalectomized-gonadectomized males, 31.0%, and similarly treated females, 25.5%.

Normal-pregnant females absorbed 51.5% and adrenalectomized-pregnant females, 63.2% of the 1 cc. of oil injected on the 10th day of pregnancy and recovered 21 days after delivery, as compared with 72.7% absorbed by virgin females after a comparable 31-day-period. It is evident that pregnancy and lactation markedly decrease the rate of absorption, whereas a super-imposed adrenalectomy significantly increases absorption during pregnancy and lactation.

The application of these findings to the specific hormones involved in absorption of subcutaneous sesame oil, in addition to the rate of hormone-depletion from the oil deposits under different experimental conditions, is under investigation.

TORPIN, R., see Allen, L.

TROMLY, R. G., see Curl et al.

VAN CLEAVE. *The effect of low temperature on neural induction in amphibian ectoderm.* C. D. Van Cleave, Department of Anatomy, University of North Carolina.

Ectodermal explants of early Amblystoma punctatum gastrulae, with and without implants of dead optic vesicle, were isolated at a temperature of 50°F. On microscopic study, thirty-three explants, exposed to the implant for 30 days at this temperature showed: 51% epidermis only, 15% low-grade, unorganized neural tissue, 33% brain masses. A similar series of 47 explants at room temperature showed respectively 25%, 29% and 46%. Both series of ectodermal controls differentiated as epidermis. It is concluded that with early gastrular ectoderm no increase in neural induction is obtained by increasing the duration of the inducing influence by retarding development.

When very late gastrular ectoderm, presumptive belly epidermis, was subjected to the same conditions, inductions of brain masses were obtained in 75% of the explants which had not extruded the implant. The remaining 25% showed low-grade neural inductions. At room temperature, 20% showed inductions of neural tissue; ectodermal controls at both temperatures and experimental explants at low temperature which had extruded their implants differentiated as epidermis only. Since under the experimental conditions the expected restriction of potency in this ectoderm did not coincide with the close of gastrulation, it is concluded that this state of competence was maintained by low temperature long enough for the increased duration of the inducing stimulus to increase markedly the neural induction (compare Lopashov, '39).

VAN DYKE. *The behavior of ultimobranchial tissue in the postnatal thyroid gland of sheep.* John H. Van Dyke, Department of Zoology, Cornell University, and Department of Anatomy, Washington University School of Medicine.

Ultimobranchial tissue was clearly demonstrable in both lobes of the thyroid gland removed from thirty yearling sheep. It was usually represented by large multiple cysts lined by stratified squamous epithelium which proliferated cords or clumps of cells that invaded the neighboring thyroid parenchyma. Certain cells, derived from the more superficial cells of the cyst epithelium, produce other cysts lined by columnar cells (ciliated) which may undergo stratified squamous metaplasia or degenerate. Other large, gland-like cells arise in clumps from the germinative cells of the original cysts and come to lie between the cyst wall and its connective-tissue capsule. Groups of these clear cells, quite conspicuous in thyroids showing hyperplasia, form lobules that plainly differentiate into thyroid-like follicles; they may even show the involution characteristic of hyperplastic thyroid parenchyma. Gland-like cells sometimes persist as interfollicular clusters or stimulate a lympho-epithelial reaction. Two aberrant thymi (IV?) have been identified. The evidence is interpreted as indicating that cyst epithelium, indicative of ultimobranchial tissue which lacked inherent thyroid forming potencies, is capable of producing indifferent gland-like cells which may be induced by adjacent thyroid parenchyma to transform into thyroid-like follicles. These cysts or those formed anew upon occasion from thyroid-like follicles may produce new follicles during thyroid hyperplasia, a condition previously reported in vitamin A deficient rats.

VICARI. *Cortical lipids of the adrenal gland of mouse strains with different tumor incidences.* E. M. Vicari, Roscoe B. Jackson Memorial Laboratory.

The object of this work is to determine whether a difference in the amount of cortical lipids of the adrenal gland is associated with incidence of mammary tumors. Animals used were mice from six inbred strains at the Roscoe B. Jackson Memorial Laboratory. This report deals with the 34-day-old age group of these animals.

The adrenals of approximately two-hundred individuals have been studied by the Romeis Sudanophile method and the cholesterol positive reaction test for histological sections.

The results show a variation in distribution of lipids from ninety-eight — 60% of the total cortical width. Two zones, one wider, heavily laden and one narrower, less dense, contain lipids. A third zone is free from lipids. The six strains arrange themselves in order of amount of lipids with the largest amount first mentioned; strains C 57 Black, N, ce, (low tumor incidence), A, (low tumor incidence in virgins, high in breeders), C3H and dba strains (high tumor incidence). Accessory adrenal glands are found most frequently in the stocks with greatest amount of cortical lipids in the normal gland.

Studies are being conducted on other age groups and on hybrids between these strains.

WAGNER, V., see Knouff et al.

WARD, et al. *Electrical stimulation of the caudate nucleus following acute and chronic lesions involving cortical projection systems in cats.* James W. Ward and Jesse Scott, Department of Anatomy, Vanderbilt University Medical School.

The caudate nucleus was stimulated with sine-wave currents acutely and chronically in normal cats, after removal of the frontal poles of the cerebrum; chronically after section of one basis pedunculi, and after certain combinations of these two operations. Results: (1) Discrete movements can be elicited from specific regions in the head of the caudate of normal unanesthetized cats (anterior-inferior portion — head to opposite side, middle region — contralateral fore-leg lifting and head movement, posterior-superior region — contralateral posterior and anterior leg lifting). (2) Immediately following the removal of a frontal lobe exposing the caudate, similar movements were obtained by stimulation of the exposed caudate. (3) In unanesthetized cats stimulation of the caudate by permanent electrodes planted several weeks after the removal of a frontal lobe on the same side failed to elicit such activity. (4) Discrete movements were obtained acutely when the caudate was stimulated in animals whose basis pedunculi had been sectioned on the same side months before. (5) No evidence of inhibition was seen in any of these experiments.

The responses were clear-cut. It would appear from the above that the impulses elicited in the caudate and responsible for these specific responses were conducted antidromically in the collaterals of the internal capsule and from there were transmitted on down to some lower lying ''extrapyramidal center'' which projects to primary motor neurons contralateral to the stimulated caudate nucleus.

WARD, J. W., see Chambers

WARD, J. W., see Clark, S. et al.

WIERDA. *The occurrence of colonic diverticula in rats which were fed a high-fat diet.* Jay L. Wierda, University of Pennsylvania. (Introduced by Roy G. Williams.)

Three successive generations of white rats were fed a diet consisting of 55% fat (by weight). After 25 to 55 weeks these rats were killed and the digestive tract studied. Thirty litter-mates were fed either a balanced, a high-carbohydrate, or an agar-supplemented diet for 32 to 50 weeks. Studies of the intestine showed no changes or abnormalities other than deviations in dimensions (Wierda, '42). Three rats of the third generation were kept on the high-fat diet for 90 to 111 weeks, and all three showed large diverticula of the colon at autopsy. Each rat had a large diverticulum near the caecum, and in one there was also a diverticulum on a lower segment of the colon. The wall of each diverticulum included a representative of each of the layers of the colon. Studies on the richness of the connective tissue of the colon in elastic fibers showed that the colon wall of the rats which developed diverticula was as rich as the colon wall of rats of the same age which had not developed diverticula. It was found that elastic fibers are as abundant in the proximal part of the colon as in the distal part, so the more frequent occurrence of diverticula on the proximal colon cannot be explained on that ground.

WIGGINTON, R. C., see Eliot et al.

WILLIAMS, R. *Observations on certain non-thyroid cells around the follicles of the thyroid gland in mice.* Roy G. Williams, Department of Anatomy, University of Pennsylvania.

Vital and post-vital injections of methylene blue into the vessels of the thyroid gland in mice frequently stained nothing but large cells distributed around the follicles throughout the body of the gland and at its periphery. These cells are characterized by their large, irregular size, small, deeply-staining and frequently eccentric nuclei, and vacuolated cytoplasm, the vacuoles being surrounded by fine, blue granules. The cells resemble those in the zona fasciculata of the suprarenal gland. They also resemble brown fat cells. The masses of brown fat in the neck or elsewhere did not stain when subjected to the dye simultaneously with the thyroid. These are the only cells so far identified, other than those in connective tissue and endothelium, not a part of follicles in the thyroids of mice and are not, apparently, the parafollicular cells of Nonidez. The very close morphological similarity between these cells found in the thyroid gland, the ''brown fat'' cells and the cells of the zona fasciculata of the adrenal suggests a possible relationship between them.

WILLIAMS, R. G., see Wierda

WILLIAMS, W. L. *Effect of prolactin on the post-parturient unsuckled mammary gland of the mouse.* W. Lane Williams, Departments of Anatomy, The University of Rochester, School of Medicine and Dentistry, and Yale University School of Medicine.

Twenty uniparous CHI (Strong) mice were segregated from their litters at parturition and prior to any suckling. Ten mice received daily subcutaneous injections of 2 mg. (20 mg. per cubic centimeter in water at Ph 8) of prolactin (Difco) for 4 to 10 days subsequent to parturition. These animals as well as untreated ones were killed for study at daily intervals during this time. Mammary glands were studied as whole mounts and in sections. The mammary glands of the untreated animals ceased secreting within 2 days post partum and parenchymal involution was completed within 6 days. The mammary tissue of the treated animals was highly responsive to the injected prolactin. Secretion continued for 7 days and the parenchymal involution was strikingly retarded. Full lactation did not occur and the parenchyma gradually involuted after the fifth day. However, the treated animals showed a greater degree of secretory activity and of parenchymal maintenance on the tenth day post partum than did the controls on the fourth day. Retention of secretion was not sufficient to cause any considerable intraglandular stasis and concomitant inflammation. Neutrophiles and macrophages were present in the lumina of ducts and alveoli. Parenchymal regression was accompanied by a rapid growth of the fatty stroma. The prolactin was effective in prolonging secretion in the absence of suckling as well as in retarding parenchymal involution.

WILLIAMS, W. L., see Dougherty et al.

WILSON. *Reproductive capacity in males treated prepuberally with sex hormones.* James G. Wilson, Department of Anatomy, The University of Rochester, School of Medicine and Dentistry. (Introduced by Karl E. Mason.)

Eight male rats were injected thrice weekly over the first postnatal month with .75 to 36 mg. of testosterone propionate and eighteen rats were injected during a corresponding period with .037 to 2.4 mg. of estradiol dipropionate.

Permanent impairment of reproductive capacity was evident when the animals were studied three months later. Libido, determined by observing mating behavior with estrous females during repeated 15-minute test-periods, and fecundity, determined by caging with fertile females for 6 or more cycles, were absent or greatly reduced compared with the libido and fecundity of normal males tested similarly. Only one animal, receiving the smallest dose of androgen, sired a litter and consistently mounted estrous females.

The weights of testes and accessories were markedly subnormal following treatment with either hormone. The seminiferous epithelium in estrogen-affected males was destroyed or severely damaged, the tubules usually containing only Sertoli cells. Conversely, the seminiferous epithelium in androgen treated males was intact; and the presence of motile spermatozoa in the vas deferens at autopsy indicated that the deficient fecundity in these rats was due to lack of libido. These observations, together with unpublished data, suggest that estrogen administered at an early age suppresses the production of the pituitary gonadotropic factor responsible for maintenance of the germinal epithelium; whereas androgen acts on the testis in a manner, as yet unexplained.

WINDLE, et al. *Structural alterations in the nervous system resulting from anoxia at birth.* W. F. Windle and R. F. Becker, Institute of Neurology and Department of Anatomy, Northwestern University Medical School.

Guinea pigs were asphyxiated before birth by clamping uterine or umbilical vessels after delivering littermate controls. After intrauterine respiratory movements, induced by anoxia, ceased and heart beats became weak, the animals were delivered and resuscitated by inflating their lungs. Physiologic effects of anoxia, impairment of learning ability and behavioral changes were studied. Brains were preserved, sectioned serially and prepared by neurologic methods.

High incidence of cytopathology was found. Twenty-two specimens were anoxic for 10 or more minutes and manifesting cardiac impairment, spastic states, tremors, incoordination, loss of certain reflexes, etc., for a day or more. All showed structural alterations in the brain to varying extent and severity. In early hours after anoxia, marked vascular congestion and fortuitous capillary hemorrhages were found. By 24 to 30 hours, cellular swelling and chromatolysis appeared. Edema occurred between 2 and 4 days, ventricles becoming dilated. Glial proliferation was observed at 3 to 9 days. Disappearance of nerve-cells was detected after 4 days. Atrophy, sometimes localized, sometimes generalized and marked, was evident after 9 days. The cerebral cortex appeared to be most readily affected, but other regions, except the cerebellum, showed fortuitous damage. Brain atrophy resulting from anoxia was not always correlated with permanent behavioral changes or failure of learning ability.

WINDLE, W. F., see Rhines

WISLOCKI, et al. *Foamy, lacunar, vacuolated and vesicular appearances of the syncytial trophoblast in the normal placenta of man and rhesus monkey.* George B. Wislocki and H. Stanley Bennett, Department of Anatomy, Harvard Medical School.

Vacuoles occur normally in a variety of forms in the syncytial trophoblast of the primate placenta: (a) vacuolization of the syncytium at the time of implantation, leading to formation of the primary lacunae; (b) variably occurring, fine-grained, non-lipoidal vacuolization and foamy appearances of the syncytium; (c) larger vacuoles in the syncytium covering the cell-columns during the third week of development (macaque); (d) larger vacuoles in various parts of the syncytium, especially that associated with the trophoblastic shell, cell-columns and cell-islands.

We attribute the formation of all of these types of vacuoles, during normal development, to taking up or imbibition of fluid from the implantation site or the intervillous space. Vacuolization manifests itself preponderantly in the period before the secondary villi and the chorionic circulation are established and persists longest in the syncytium, associated with the trophoblastic shell, cell-columns and cell-islands, where fetal capillaries are absent. At implantation the vacuoles in the syncytium coalesce and break down, forming the primary trophoblastic lacunae. In later stages vacuolization diminishes in those localities where the fetal circulation provides for adequate transmission of fluid to the fetus. In the outlying syncytium the vacuoles tend to persist, but as this syncytium ages and degenerates, the vacuoles disappear and their contents are evacuated into the intervillous space. A similar genesis of hydatidiform moles and of the primary lacunae has been predicated by Hertig and Edmonds ('40).

WOLFE. *Effects of advancing age on the structure of the anterior pituitary of the rat.* J. M. Wolfe, Department of Anatomy, Albany Medical College, Union University.

The anterior pituitaries of one hundred and sixty-six virgin rats ranging in age from 1 to 28 months were studied. Structural alterations, which were correlated with advancing age, occurred. There was a progressive decrease, which was most marked early in life, in the percentages of the eosinophiles and an increase in those of the chromophobes. Mitoses which were found in the eosinophiles and chromophobes were most abundant in the immature rats; they declined rapidly and were found infrequently in rats older than 6 months. A degenerative process in which the anterior lobe cells were replaced by acidophilic colloid, and the appearance of intercellular and cleft colloid were first noted in the 3-month-old rats. These reached a maximum in rats 6 to 12 months of age and then remained stationary. Vacuolated basophiles were first observed in the anterior lobes of rats 3 months old; they increased slowly as age advanced but never became abundant. Adenomatous changes occurred in the anterior pituitaries of 27.8% of the rats 17 months of age and above. Basophiles, predominantly granular, were most abundant in the immature rats; they were less numerous in the mature rats and their number was not further altered by advancing age. In many old rats there was a decrease in the histologic evidence of secretory activity in the anterior lobe cells.

WOLFE. *Effects of advancing age on the structure of the ovaries of the rat.* J. M. Wolfe, Department of Anatomy, Albany Medical College, Union University.

The ovaries of one hundred and thirty-six virgin rats ranging in age from 3 to 28 months were studied. As age advanced definite structural alterations were found. Normal follicles, 0.3 mm. in diameter and above, were most abundant in the ovaries of rats 3 months old; the number of these decreased progressively throughout the life span, most rapidly early in life. Atretic follicles, 0.3 mm. in diameter and above, were also most abundant in the 3-month-old rats; their number was sharply reduced in rats 6 months old and remained low (below the level of normal follicles) until late in life when there was a pronounced increase of follicular atresia so that atretic follicles became more abundant than normal follicles. Corpora lutea remained fairly constant in number from 3 to approximately 20 months of age; they then rapidly became less numerous as age advanced further. Failure of ovulation, the formation of wheel cells, and cystic follicles were first observed in the ovaries of rats 12 months old and became increasingly prevalent as age increased. Interstitial tissue was present in variable amounts throughout life, but was definitely increased in the older rats. Spontaneous vaginal mucification was noted frequently in rats 25 months of age and above.

WOLFSON, C., see Latimer et al.

WORTHINGTON, R., see Allen, E.

YEAKEL, E., see Farris, E.

ZEIT, W., see Carey et al. 1 and 2

ZIMMERMANN. *The arterial pattern in the fore- and hindlimbs of the opossum.* Arnold A. Zimmermann, Department of Anatomy, University of Illinois, College of Medicine.

In the forelimb the main features reside in a well-developed a. brachialis superficialis, an a. mediana as preponderant vessel of the forearm, a rudimentary a. ulnaris and absence of both a deep radial artery and volar arch. The superficial brachial artery divides into the superficial ulnar, volar intermediate and superficial radial arteries. The latter gives off an a. dorsalis manus superficialis. The brachial artery passes through the entepicondylar foramen with the median nerve. The deep ulnar artery arises on a common stem with the common interosseous vessel and terminates in short muscular branches. The interosseous arteries do not reach the carpus. The a. mediana forms four aa. digit. communes. In the hindlimb superficial arteries again are characteristic. They derive from the a. saphena and from descending branches of the a. femoris profunda. The saphenous artery gives off the a. genu suprema and divides into the primary a. tibialis posterior and into the a. dorsalis pedis superficialis. The former continues into the tarsus as a. plantaris which forms four aa. digit. communes. The descending branches of the profunda femoris reach the dorsum of the foot as superficial peroneal vessels. The popliteal artery forms the a. tibialis anterior which continues into the foot as a. dorsalis pedis profunda. The dorsal branch of the popliteal artery continues distad as truncus peroneotibialis.

HEMOPOIESIS IN THE CHORIONIC VILLI OF THE PLACENTA OF PLATYRRHINE MONKEYS[1]

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ONE TEXT FIGURE AND TWO PLATES (EIGHT FIGURES)

Professor J. P. Hill ('32), in his Croonian Lecture delivered in 1929, announced the discovery of erythropoiesis in the placental chorionic villi of platyrrhine monkeys. His observations were embodied in a very brief but concise description accompanied by drawings. He stated that in association with embryos of platyrrhine monkeys (Chrysothrix, Hapale, Cebus, Mycetes) of several centimeters or more in length he encountered " a very remarkable, possibly unique, phenomenon in the platyrrhine placenta and that is the formation inside enlarged capillaries situated in the mesenchymal axes of the villous branches, of foetal non-nucleated red blood corpuscles from parent cells, identical in character with those from haemopoietic centres." Although unrecorded at the time by either title or text, Hill ('32) remarks that his preparations showing erythropoiesis were exhibited at the Liége meeting of the Association des Anatomistes in 1926.

The present writer ('30 and '32) mentioned briefly the presence of hemopoietic foci, associated with dilated fetal vessels in the chorionic trabeculae of several placentae of the spider monkey, howling monkey and the marmoset, but failed to identify these specifically as erythropoietic centers.

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[1] The placental material utilized here was obtained in Panama in 1937 under a grant from the Committee on Sex of the National Research Council. For aid in obtaining the specimens the writer is greatly indebted to Mr. J. B. Shropshire. My sincere thanks are due to Dr. James Zetek and Dr. Herbert Clark who placed the facilities, respectively, of the Barro Colorado Island Laboratory and the Gorgas Memorial Institute at my disposal.

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Recently, however, I have examined a series of placentae of marmosets, and have been able to substantiate Hill's interesting discovery and the correctness of his interpretation. Study of this new material has yielded certain additional information regarding the nature of these hemopoietic foci. In connection with the question of the placental hemopoiesis, the livers and spleens of two marmosets have been examined with interesting results. In view of the outcome of the present study, I regard it as justifiable to review briefly this subject and to present my additional observations.

MATERIAL AND METHODS

The present material consists of ten placentae of marmosets (Oedipomidas geoffroyi) obtained from uteri containing fetuses which ranged from 15 to 90 mm. in crown-rump length. The placentae were fixed in 10% formalin at periods varying from 2 to 5 hours after death. Sections were prepared and stained in a variety of ways, including hematoxylin and eosin, eosin and methylene blue, phloxine and methylene blue, and Giemsa's stain.

Certain additional information was obtained from several placentae of spider monkeys (Ateles geoffroyi) and howling monkeys (Alouatta palliata) previously secured and stained in hematoxylin and eosin.

In addition to the placentae, the liver, spleen and adjacent abdominal organs were examined in two marmoset fetuses which were 40 and 60 mm. in crown-rump length.

OBSERVATIONS

The vessels under consideration in the chorionic villi (fig. 2) consist principally, as Hill ('32) observed, of dilated, fetal blood vessels which are packed with differentiating erythrocytes and are scattered throughout the entire extent of the placenta. These large, thin-walled vessels, loosely filled with deeply staining, nucleated blood cells, are very conspicuous. Besides these dilated vessels, the villi also contain numerous much smaller, inconspicuous capillary vessels, which contain, mainly, ordinary non-nucleated erythrocytes. In the absence of injected preparations, or reconstructions, of the vascular bed of a typical villus, one can only surmise as to the relationship of the hemopoietic vessels to the general vascular tree. It seems logical to assume by analogy with the blood vessels in the human chorionic villus, that the vessels form a network. In this plexus evidently the blood channels are of two kinds: (1) ordinary capillaries, in which, judging from the presence of mature erythrocytes, the blood must be circulating and therefore must be regarded as subserving the general nutritional and metabolic needs of the placental tissue; and (2) dilated channels, fewer in number, in which erythropoiesis is occurring and the blood cells are not circulating. Thus the latter constitute blood vessels which must be connected anatomically with the plexiform vascular tree of the villus, yet are functionally closed. They probably release their contained cells, when these have matured, into the general vascular bed with which they anastomose and in which blood is circulating. Hill refers to these erythrogenic vessels as dilated umbilical capillaries. Yet, because of their great size, the extreme thinness of their walls, and the fact that the endothelial cells lining them are swollen and appear to be engaged in the production and liberation of undifferentiated blood cells, they possess the features of vascular channels which elsewhere in the body are designated either as sinusoids (liver) or as venous sinuses (spleen). Thus I hazard the surmise that in the anastomotic capillary bed of these chorionic villi certain segments or links between the capillaries, or near the venous end of the bed, have become modified to form erythrogenic sinusoids or sinuses. These sinuses are relatively extremely large, and hence they bulge on the sides of the villi into the intervillous space, in consequence of which they appear to be separated from the maternal blood stream by only a very thin layer composed of syncytial trophoblast, a modicum of reticulum, and endothelium (figs. 1 and 2).

The cells contained in the lumen of these sinusoidal vessels consist of various stages of maturing erythrocytes, as Hill observed. Most conspicuous are large, free stem-cells with deeply basophilic cytoplasm (figs. 1 and 4), which Hill designates as hemocytoblasts. Derivative from these large, antecedent, basophilic cells one observes erythroblasts, normoblasts and erythrocytes. In erythrogenic sinusoids which are cut at random the picture varies from sinusoid to sinusoid.

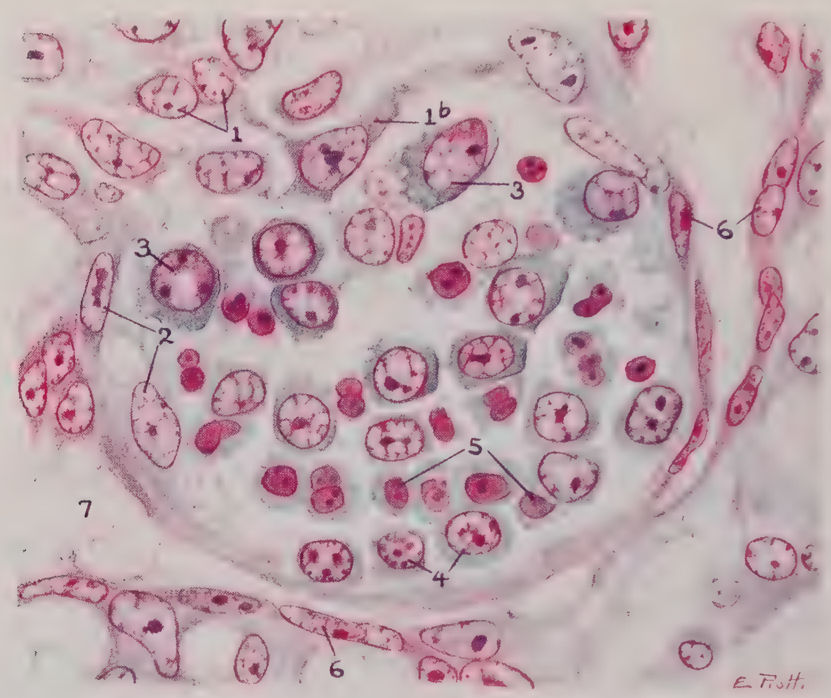

Fig. 1 A drawing of an erythrogenic vascular sinusoid in a chorionic villus of the placenta of a marmoset (Oedipomidas geoffroyi, crown-rump length, 40 mm.). Observe the sinusoid bulging into the intervillous space, with the thin, separating membrane composed of syncytial trophoblast and endothelium. Above, the wall of the sinusoid appears to be incomplete, suggesting that endothelial cells (angioblasts) and free stem-cells are differentiating directly from the mesenchyma. The atypical bluish staining of the cytoplasm of the erythroblasts and normoblasts is presumably due to the formalin fixation which has probably dissolved much of the hemoglobin. 1, mesenchymal cells; 1 b, mesenchymal cell, or angioblast, transforming into free stem-cell; 2, endothelial cells, one of which (lower left) is almost completely detached from the vessel wall; 3, free stem-cell; 4, erythroblast; 5, normoblast; 6, syncytial trophoblast; 7, intervillous space. Eosin-methylene blue. × 1500.

In some, the cells are almost exclusively of the large, basophilic variety (fig. 4); in others, cells in various stages from the large, blue cells through erythroblasts to normoblasts are encountered (figs. 1 and 5), while in still other cross-sections normoblasts and erythrocytes dominate the field. This indicates that in these vessels different phases of erythropoiesis predominate in various segments. The atypical bluish staining of the cytoplasm of the erythroblasts and normoblasts is presumably due to the formalin fixation which has probably dissolved much of the hemoglobin.

Besides the maturing red blood cells, megakaryocytes are frequently encountered. These may occur within the erythrogenic sinusoids, and also in what appear to be ordinary capillaries, as well as occasionally interstitially, outside of the blood vessels in the mesenchymal stroma (fig. 3). The nuclei of these megakaryocytes impress me on the whole as being rather simple in form, without elaborate lobation.

In the erythrogenic sinusoids, occasional monocytes with characteristic, small, indented, or bilobed, nuclei have been observed, as well as, more rarely, larger cells of the same variety, resembling macrophages.

The endothelial cells lining the sinusoids are usually very conspicuous; many of the endothelial nuclei are large and are accompanied by an excess of cytoplasm, so that the cells appear swollen and bulge distinctly into the lumen. Some of the swollen endothelial cells acquire a more basophilic cytoplasm and appear to be in process of detaching themselves from the vessels to become the large free stem-cells (hemocytoblasts) encountered in the lumen.

The impression is gained, however, that the walls of the dilated sinuses are not always completely lined by endothelium. Instead, in certain places, the loose mesenchyma of the villus appears to abut directly on some part of the vessel lumen, suggesting that the walls of some of the erythrogenic sinusoids are incomplete and that in such localities their walls are in process of differentiating in situ from the adjacent mesenchyma (figs. 1, 4 and 5). In these positions the abutting mesenchyma appears to be differentiating into endothelial cells (angioblasts), as well as possibly directly into free basophilic stem-cells (hemocytoblasts) (fig. 1). In such places where the vessels are seemingly in process of differentiation, free stem-cells which appear to derive directly from mesenchyma, may become located in extravascular spaces which are not clearly a portion of the vascular lumen. In such areas it is sometimes difficult to decide whether the free cells have arisen strictly inside or outside of blood vessels. It is clear that the pattern of erythrogenesis described here bears a marked resemblance to that observed in the blood vessels of the wall of the human yolk-sac as outlined by Bloom and Bartelmez ('40).

The above interpretation appears to me to fit most nearly the appearances seen in the sections. Yet, it is possible that some of the free stem-cells (hemocytoblasts), here involved, may have conceivably been carried to the placenta through the fetal circulation from other hemopoietic foci in the fetus, thus becoming lodged in the chorionic capillaries where they have undergone division. That all of them could originate in that way seems unlikely in view of the histology of the vessel walls. Another point of doubt concerns the gaps in the walls of the sinuses where differentiation of endothelium (angioblasts) and free stem-cells appears to be occurring directly from mesenchyma. This involves the consideration as to whether at this relatively late period the vessels in the chorionic villi are capable of growing at some points by the direct differentiation of mesenchyma into angioblasts. One might proceed on the assumption that vessels of this size and age should be completely closed and that all growth should occur by direct extension of previously differentiated angioblasts without the direct participation of mesenchyma. If this were the case, the gaps occurring in the walls would have to be regarded as artifacts produced in the course of obtaining and fixing the material. Yet, in spite of the fact that the placental material could not be fixed until several hours after death, the preparations seem on the whole to be so well preserved and stained as to preclude this possibility.

One is dealing in these sinusoids apparently with the formation and differentiation of definitive, non-nucleated erythrocytes and not with the primitive red blood cell series encountered in the embryonic adnexa, especially in the wall of the yolk-sac, in the very earliest stages. Hill remarks that the appearance of the blood-forming foci in the chorionic villi "would seem to coincide with the replacement of the nucleated red corpuscles of the foetus by non-nucleated, for in this stage (Chrysothrix nigrivittatus 648, length 25 mm.) such nucleated corpuscles as occur in the large umbilical vessels are few in number as compared with the non-nucleated and, judging from the condition of their nuclei, are in process of degeneration." Wislocki ('32) also remarks that the umbilical vessels and their branches contain no such large proportion of nucleated cells as the hemopoietic sinuses display.

Regarding the time during the period of gestation that hemopoiesis occurs in the chorionic villi, in the manner outlined, Hill describes it specifically in the placenta of a 25-mm. embryo of Chrysothrix nigrivittatus, as well as also in an 82-mm. fetus of Cebus gracilus. In a specimen of Cebus macrocephalus with an embryo of 10 mm., blood-forming foci were not established, according to Hill. Without further mention of specific lengths, he concludes that the phenomenon is of fundamental importance because it occurs in later stages and is present in the placentae of all of the genera which he examined. My experience has been similar. I have observed it in marmosets (Oedipomidas), spider monkeys (Ateles), and howling monkeys (Alouatta). It is not present in a 16-mm. marmoset in my series, but can be characteristically seen in nine other placentae of marmosets ranging from 35 to 90 mm. in crown-rump length. It is also present in the chorionic villi of a spider monkey measuring 48 mm., besides two howling monkeys of 43 and 60 mm. The phenomenon evidently ceases in later stages, a point which Hill does not specifically clarify. In placentae of six fetuses of Alouatta and Ateles, ranging from 120 to 195 mm. in crown-rump length, I have found no evidence of erythrogenic sinusoids. Thus, placental hemopoiesis of the character described here is a feature of the mid-gestational period involving perhaps roughly the second and third quarters of pregnancy. It subsides probably just before or at the period when the fetus becomes viable, that is, at the time that it first becomes possible for the fetus to survive, if obliged to, outside the mother's body. Obviously, survival outside the uterus requires the presence of fetal hemopoiesis in organs (liver, spleen, marrow) other than the placenta which is only an adnexa of the growing organism.

In connection with this point I have examined the liver and spleen of two marmosets, 40 and 60 mm. in crown-rump length respectively. In the youngest the liver is uniformly of the character shown in figure 6, without any hemopoiesis whatsoever. In the older specimen, the large bulk of the liver is also devoid of hemopoietic activity, as revealed in figure 7. In the latter, however, there are areas containing blood channels, as shown in figure 8, which are crowded with primitive stem-cells (hemocytoblasts) in process of producing erythrocytes. Hemopoiesis is, however, not taking place diffusely in the walls of the hepatic sinusoids, but is definitely localized in certain of the radicals of the hepatic veins. In figure 8 an hepatic vein is observed with tributary sinusoids, a few of which at the point of entry into the vein are dilated and packed with developing cells. These cells are apparently being discharged from the mouths of the tributaries into the main venous channel. This picture is unlike ordinary hemopoiesis as described in the fetal liver. I have examined a number of fetuses of man, pig, rabbit and cat in the Minot Embryological Collection and have found hemopoiesis in the livers of these animals to be rather constant in character, consisting of a uniformly and diffusely distributed hemopoietic process, occurring rather generally throughout the hepatic lobules in the walls of the sinusoids. The hemopoiesis in this older marmoset specimen is quite unlike the process observed in these other animals and raises some doubt as to whether the cells segregated in clumps in certain of the hepatic vein radicals are arising in situ from actual cellular activity in the contiguous vessel walls.

Regarding the spleens of the two marmoset fetuses, they are rather small in volume. Figure 9 shows the spleen of the youngest specimen and it is in every respect similar to that of the older fetus. It gives the impression of being quite cellular, possessing a framework of large, fixed cells, besides free cells in its interstices. The cells appear to be almost exclusively of a primitive type with large nuclei and basophilic cytoplasm and do not appear to be differentiating to any great extent into smaller, hemoglobin-bearing derivatives.

The neighboring kidneys which were cut along with the liver and spleen show no hemopoiesis. The adrenal glands of both fetuses, however, contained in the same sections, show numerous dilated venules in the medullary region, which are packed with primitive cells and differentiating erythrocytes, bearing a striking resemblance to the venules packed with cells observed in the liver of the older marmoset fetus.

These observations on the liver and spleen suggest that, in the marmoset, placental hemopoiesis substitutes in large measure, during the first half of gestation, for the usual sites in which in other animals erythropoiesis occurs. Yet, as pointed out above, since the placenta is an adnexa lost at birth, and since it loses its hemopoietic function in the second half of gestation, its role as a blood-forming organ must eventually be taken over by centers in the fetus itself.

The fact that these erythrogenic sinusoids are not encountered until an age after the circulation is established does not imply that in the earlier embryonic period, when the vascular system is forming, blood cells may not arise to some extent in the chorion. Hertig ('35) does not exclude this as a possibility in early Primate ova, although Bloom and Bartelmez ('40), studying human material, find little evidence of erythropoiesis in the fetal membranes, other than in the mesenchyma and vessels of the wall of the yolk sac. In any event, the peculiar, conspicuous, hemogenic vessels of the chorionic villi of platyrrhine monkeys, under consideration here, do not appear until the circulation is well established and the embryos have reached a length of two or more centimeters.

With the possible, but unlikely, exception mentioned above, of hemopoiesis in the chorion in the earliest stages of development, I agree with Hill who "believes this to be the first authentic record of the normal occurrence of haemopoiesis or more strictly erythropoiesis in the mammalian placenta." Certainly, as Hill points out, Hubrecht's (1898) contention that in the placenta of Tarsius and Tupaia maternal erythrocytes are formed by the fragmentation of trophoblastic nuclear masses does not seem convincing. In human material, from a case of congenital hydrops, Goormaghtigh ('25) has described erythroblastic proliferation, while more recently Hellman and Hertig ('38) similarly describe intracapillary erythropoiesis in a series of human placentae associated with erythroblastosis and congenital hydrops of the fetus. They remark that in the villi with hyperplastic stroma there are many foci of intracapillary erythropoiesis and that the endothelium of the vessels consists of large cells whose nuclei project into the lumens of the vessels. Hellman and Hertig's illustrations of this pathological condition indicate that the erythropoiesis in a given villus involves all of the capillaries about equally, instead of being confined to a special group of sinusoids to which the process is essentially limited, as is the case in the normally occurring process in the placenta of platyrrhine monkeys.

SUMMARY

Confirming a discovery by J. P. Hill, erythropoiesis is observed in certain dilated blood vessels of the chorionic villi of the placenta of the marmoset and other platyrrhine monkeys.

The present investigation indicates that the thin-walled, dilated vessels in the chorionic villi are of the nature of sinusoids or venous sinuses forming part of the vascular net of the chorionic villi. The basophilic, free stem-cells in these

vessels differentiate into definite red blood cells, and appear to originate in part from the large, swollen, endothelial cells lining the sinusoids. The preparations suggest also that in places the walls of the sinusoids are incomplete, and in such areas angioblasts, as well as free stem-cells, appear to differentiate directly from mesenchyma. Evidence for these interpretations is presented. The manner of red blood cell formation, here described, resembles very closely accounts of hemopoiesis as observed in the blood vessels of the yolk sac.

Erythropoiesis makes its appearance in the placenta of platyrrhine monkeys some time after the establishment of the circulation, but disappears completely toward the end of pregnancy.

Study of the liver and spleen of two marmoset fetuses suggests that placental erythropoiesis substitutes in platyrrhine monkeys in large measure, during the first half of gestation, for these organs which in other animals are the usual and dominant sites of blood formation during fetal life.

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PLATE 1

EXPLANATION OF FIGURES

2 Low power photograph showing the typical appearance of the erythrogenic foci associated with vascular sinuses in the chorionic villi of a marmoset. × 300.

3 Three chorionic villi, the one in the middle showing an interstitially-placed megakaryocyte (x). × 450.

4 A focus of blood formation composed entirely of basophilic stem-cells (hemocytoblasts) which appear to be arising from undifferentiated mesenchymal cells and endothelial cells in the neighborhood. × 960.

5 An erythrogenic sinusoid containing red blood cells in various stages of differentiation. On one side, the wall of the sinusoid is indistinct, suggesting that it is not completely lined by endothelium. × 960.

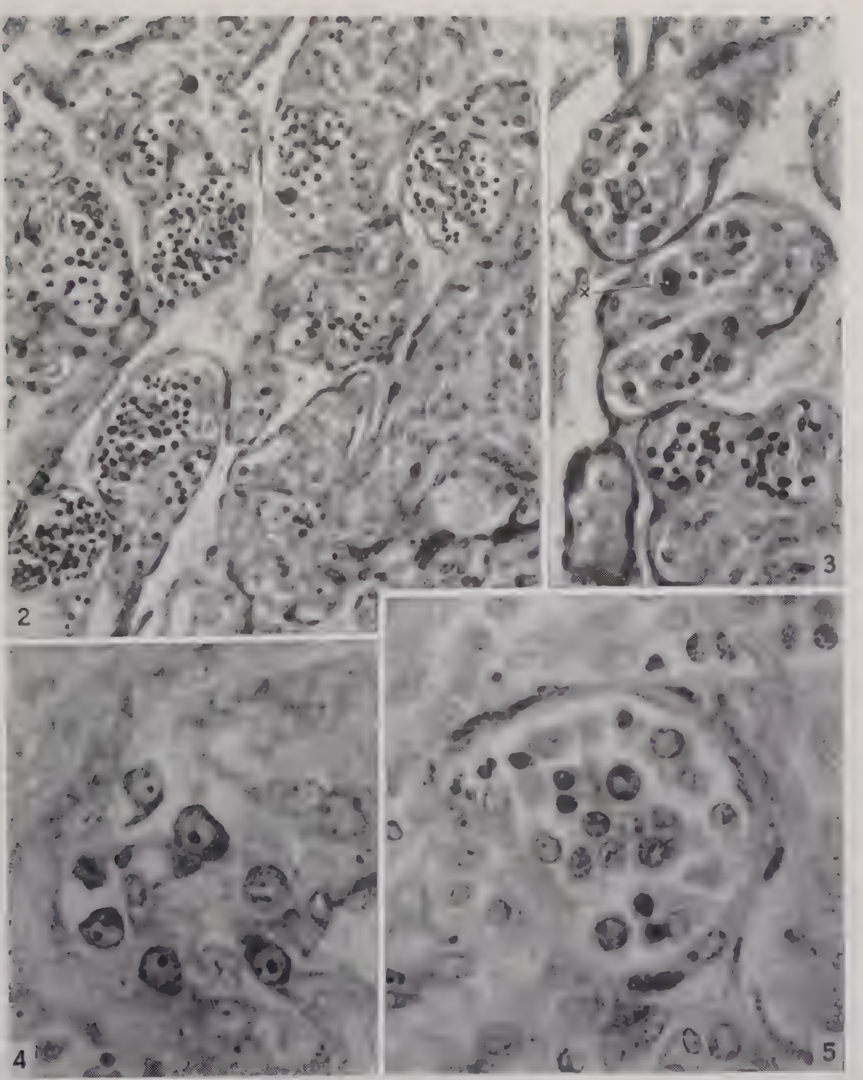
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PLATE 2

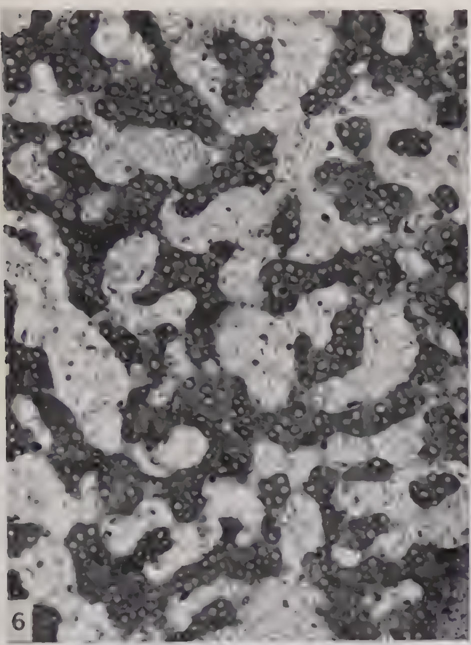
EXPLANATION OF FIGURES

6 Liver of a marmoset fetus of 40-mm. crown-rump length, showing the total absence of erythropoiesis in the hepatic sinusoids. × 220.

7 Liver of a marmoset fetus of 60-mm. crown-rump length, showing the absence of hemopoiesis in the typical sinusoids at this age. × 220.

8 A portion of the liver from the preceding specimen, in which some of the tributary sinusoids leading into an hepatic vein are crowded with primitive free cells and developing red blood cells. Diffuse hemopoiesis within the hepatic lobules is not occurring. × 140.

9 A portion of the spleen showing its cellular character, with abundant fixed and free primitive cells, but with few signs of actual erythropoiesis. × 220.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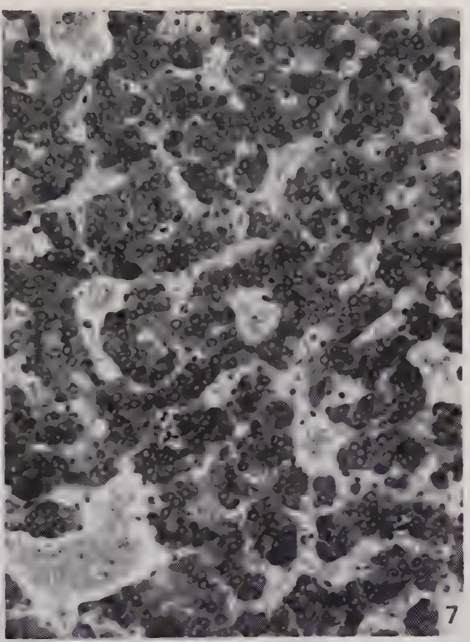
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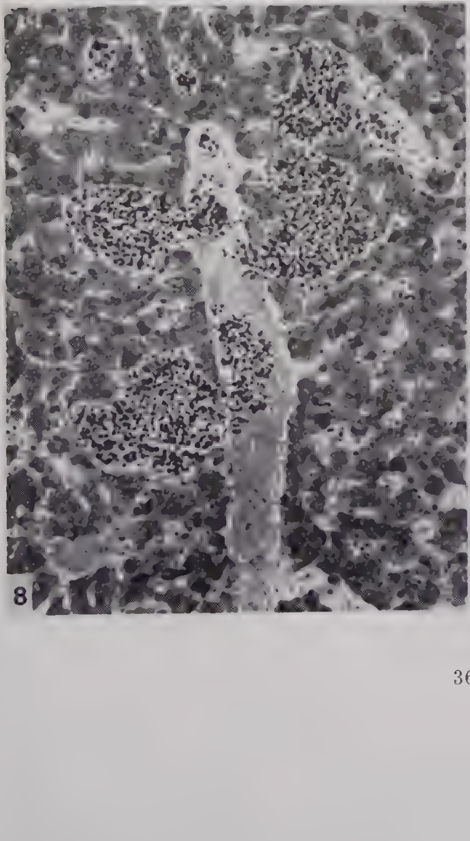
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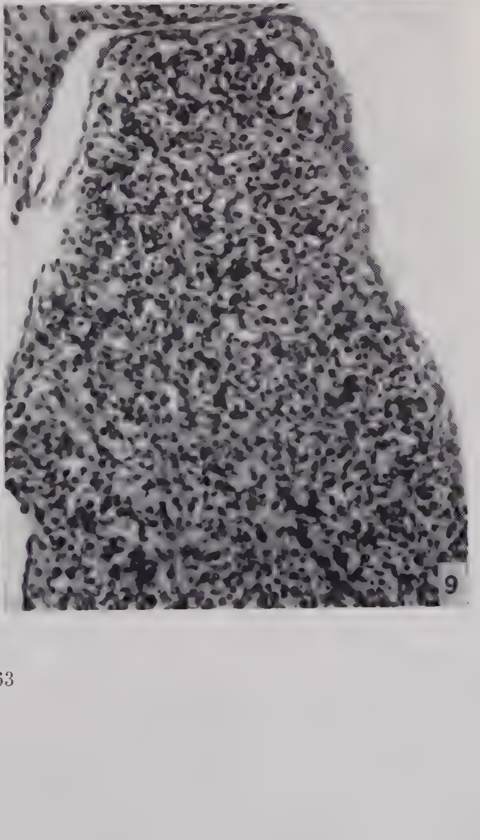
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SENSORY TYPE NEURONS IN THE HYPOGLOSSAL NERVE

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TWO TEXT FIGURES AND TWO PLATES (FIVE FIGURES)

INTRODUCTION

There are two main problems concerning the existence of a sensory component in the hypoglossal nerve. One is the presence of sensory endings of the hypoglossal nerve, and the other is the location of the sensory neurons involved. Investigators are not in agreement concerning the structural character of the sensory endings in the musculature of the tongue. Langworthy ('24), Tarkhan ('36) and others have reported the presence of neuro-muscular spindles in the tongue. Schaffer ('22, fig. 344) has illustrated in his text book a cross section of a double muscle spindle from a human tongue. A number of investigators (Boyd, '41 and others) have been unable to find muscle spindles within the tongue. The failure to demonstrate sensory endings, however, is not a conclusive proof of their absence. Weddell and others ('40) found no sensory endings in the intrinsic muscles of the tongue but report sensory endings of a tension-recording type in the genioglossus muscle. It is quite possible that the tongue has proprioceptive endings which do not possess the usual distinctive morphological characters.

After section of the hypoglossal nerve, Carleton ('38) found a complete absence of nerve endings in relation to the muscles of the tongue and concluded that if proprioceptive fibers are present, they must be located in this nerve. Barron ('36) and Corbin and Harrison ('38) were unable to record afferent impulses from the hypoglossal nerve on stretching the tongue.

Langworthy ('24), however, concluded from experimental evidence that the hypoglossal nerve in the cat carries proprioceptive impulses. Neuromuscular spindles in the tongue were found to degenerate after section of the hypoglossal nerves. Downman ('39) found that stimulation of the central stump of the hypoglossal nerve of cats caused dilation of the pupil and a rise of blood pressure. This was considered evidence of afferent fibers within the hypoglossal nerve. While many investigations fail to demonstrate a proprioceptive innervation of the tongue, the majority of authors recognize the probability of a sensory component in the hypoglossal nerve.

There has been much speculation concerning the possible course and location of sensory neurons in the hypoglossal nerve. Certain hoofed animals have persistent dorsal root ganglia connected with the caudal rootlets of the hypoglossal nerve. These are known as Froriep's ganglia. It is quite possible that the sensory neurons of the hypoglossal nerve of certain animals may have their cells of origin located in Froriep's ganglia. The occurrence of dorsal roots with ganglia of the Froriep's type in relation to the hypoglossal nerve in man is considered extremely rare (Pearson, '39). Tarkhan ('36) reported the presence of sensory ganglion cells on the peripheral course of the hypoglossal nerve in the rabbit. Carleton ('38) and Boyd ('41) have recently studied this problem and were unable to confirm this observation.

The experimental work of Corbin and others ('37) and Yee and others ('39) suggests that the hypoglossal nerve may receive a small contribution of sensory fibers from the upper cervical dorsal root ganglia. The percentages of fibers which were demonstrated with the Marchi method were very small (1–2% in the rhesus monkey and 1–5% in the rabbit). It is considered very unlikely that there is a centripetal pathway for proprioceptive impulses from the tongue by way of the trigeminal, the glossopharyngeal or the vagus nerves (Boyd, '41).

The experiments of Olmsted and Pinger ('36) are of interest when one considers the nature of a sensory component in the hypoglossal nerve. These authors found that the hypoglossal nerve, when sutured to the lingual nerve, can initiate the regeneration of taste buds where they have disappeared as a result of cutting the lingual nerve. This would suggest that the sensory neurons in the hypoglossal nerve might have functions other than proprioception.

This study is based on the serial sections of human embryos and fetuses stained by the various silver methods. The more general features of the hypoglossal nerve in human embryos and fetuses have been discussed in a previous paper (Pearson, '39).

SENSORY TYPE NEURONS

Bipolar neurons were found in the intramedullary course of the hypoglossal nerve of human fetuses of 4, 5, and 6 months of age. These neurons were spindle shaped cells with a central and a peripheral process. The clearness with which these neurons could be seen depended to some degree on the state of the preservation of the brain and the quality of the silver impregnation of the nervous tissue.

In certain of the human fetuses, small distinct swellings occurred on the rootlets of the hypoglossal nerve in the region of the hilus of the inferior olivary nucleus (fig. 1). Although these swellings were quite small, they were easily seen with the lower powers of the microscope (fig. 3). When examined with the higher powers of the microscope, these swellings were found to contain numerous spindle shaped nerve cells. These cells resembled the embryonic sensory ganglion cells of young embryos.

Spindle shaped cells occurred in many rootlets of the hypoglossal nerve which do not show a distinct swelling (fig. 4). They occurred more frequently in the more laterally placed rootlets of the nerve where the hypoglossal nerve fibers passed through the ventral wall of the inferior olivary nucleus. Many rootlets of the hypoglossal nerve appeared to be entirely free of these bipolar nerve cells. This seemed to be particularly true of the more rostrally and caudally placed fibers. The hypoglossal nerve fibers which pass along the medial side of the inferior olivary nucleus also appeared to be comparatively free from these nerve cells. The neurons in the path of the hypoglossal nerve appeared to be more numerous in certain specimens than in others. This was probably due in part, at least, to the quality of the staining of the nervous tissue. They were more easily seen in the pyridine silver preparations. It should be pointed out that these bipolar nerve cells could be demonstrated on both sides in all of the seven human fetuses of 4, 5, and 6 months of age which were available for study.

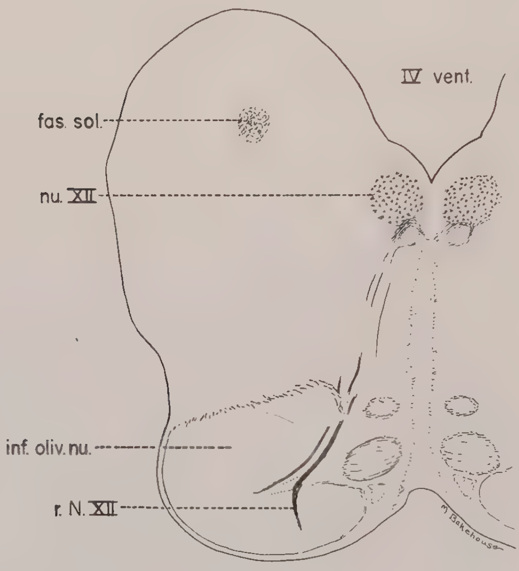

Figure 1

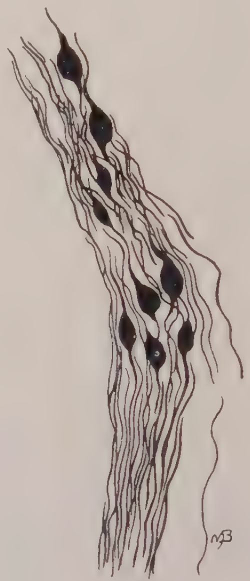

Figure 2

Fig. 1 A drawing of a cross section of the medulla oblongata at the level of the inferior olivary nucleus of a human fetus of about 4 months of age (C R 120 mm. no. 66, 9–2–4). Pyridine silver preparation. × 30. fas. sol., fasciculus solitarius. inf. oliv. nu., inferior olivary nucleus. nu. XII, nucleus nervi hypoglossi. r. N. XII, radix nervi hypoglossi. IV. vent., fourth ventricle.

Fig. 2 A bundle of fibers of the hypoglossal nerve in the region of the inferior olivary nucleus of a human fetus of about 5 months of age (no. 70, C R 160 mm.). Note the bipolar neurons among the nerve fibers. Pyridine silver preparation (16–2–1).

This would certainly suggest that their presence is not a rare or an unusual occurrence. It would indicate rather that sensory type neurons are to be expected in the hypoglossal nerve.

These bipolar cells were often scattered among the hypoglossal nerve fibers in the region of the hilus of the inferior olivary nucleus and could scarcely be seen without the aid of the higher powers of the microscope (figs. 2 and 5). In rootlets such as those shown in figures 3 and 4, the nerve cells were so closely placed that it was difficult to show their character in a photomicrograph. These cells are more easily shown in the drawing of a nerve rootlet where the cells are not too closely packed together (fig. 2). Occasionally fibers of the hypoglossal nerve could be seen which were separated from the main rootlets of that nerve. Such fibers best revealed the true structure of the neurons along the hypoglossal nerve (figs. 6 and 7). Note the spindle shape of the nerve cells and the central and peripheral processes. One nerve cell is shown at the edge of a bundle of the hypoglossal nerve fibers. There are other nerve cells present in the bundle, but these are out of focus.

Further studies are in progress to determine the origin of these bipolar neurons, their character and relationship in the adult brain, and their function.

DISCUSSION

Bipolar neurons in a cranial nerve are considered to be sensory in function. The majority of the sensory ganglion cells of the cranial and spinal nerves were probably bipolar cells at one stage of their development. At a very early period most of them become transformed into unipolar cells. Some of them (e.g., the acoustic nerve) remain bipolar cells throughout life. The question arises as to whether or not these neurons in the hypoglossal nerve remain bipolar cells.

The presence of the bipolar cells of a cranial nerve within the brain stem is an unusual situation. The presence of the cell bodies of the sensory neurons of a cranial nerve within the brain, however, is not new. It is now well established that the mesencephalic nucleus of the trigeminal nerve furnishes proprioceptive fibers for the muscles of mastication.

The problem of sensory nerve endings within the tongue musculature remains unsolved. It is generally conceded that there is a proprioceptive innervation of the tongue, and that the hypoglossal nerve is the most likely path for these fibers. The presence of sensory type neurons within the hypoglossal nerve offers the most obvious explanation as to the source of these fibers. More work will be necessary, however, to establish fully this conjecture.

SUMMARY

Bipolar neurons were found in the hypoglossal nerve of human fetuses of 4, 5 and 6 months of age. These neurons were spindle shaped nerve cells with a central and a peripheral process. They were located in the intramedullary course of the hypoglossal nerve in the region of the hilus of the inferior olivary nucleus. The character of these neurons suggests that they are sensory in function.

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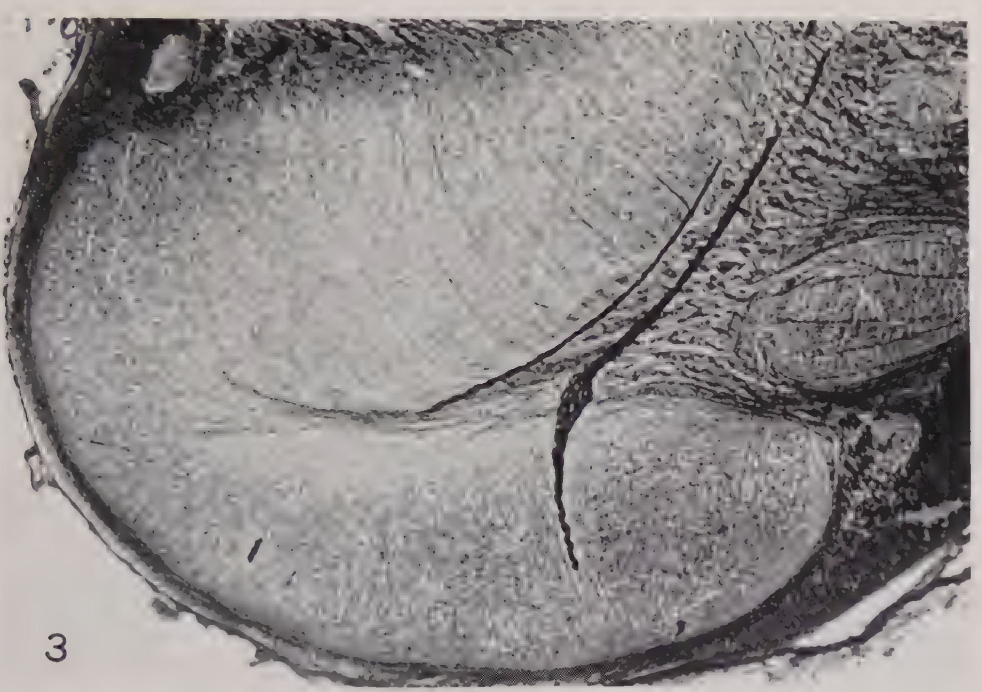
PLATE 1

EXPLANATION OF FIGURES

3 A photomicrograph of a cross section through the inferior olivary nucleus of the same section shown in figure 1. Note the slight swelling on a root of the hypoglossal nerve. Pyridine silver preparation. × 84.

4 Roots of the hypoglossal nerve in relation to the ventral wall of the inferior olivary nucleus. Bipolar neurons, though not visible at this magnification, are located among the fibers of these roots. (Human fetus no. 66, 9–3–2) Pyridine silver preparation. × 84.



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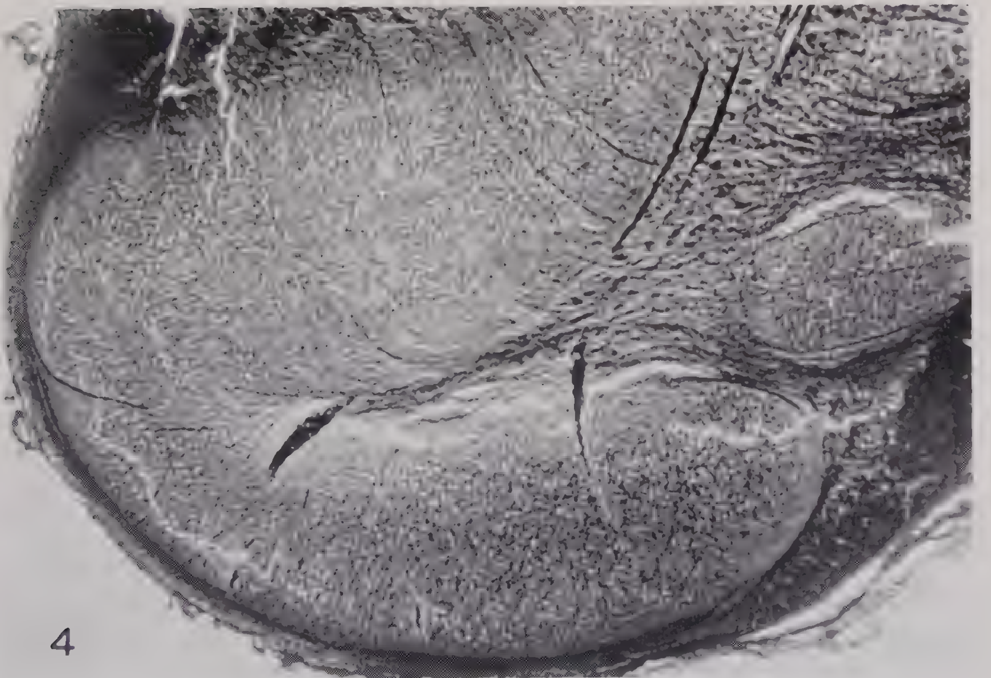

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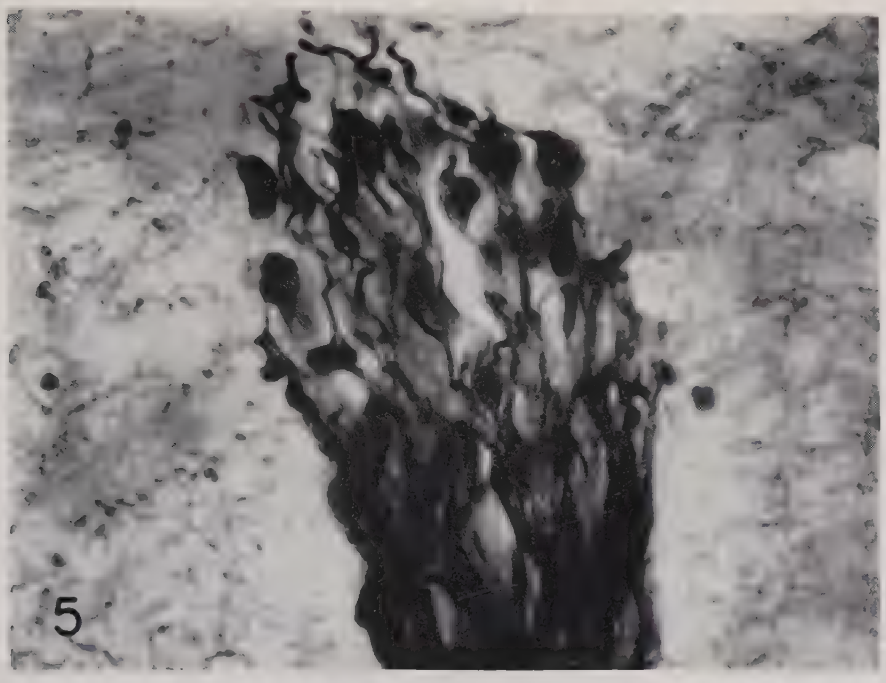
PLATE 2

EXPLANATION OF FIGURES

5 Neurons in the path of a root of the hypoglossal nerve in the region of the hilus of the inferior olivary nucleus of a 5-month-old human fetus (no. 70). Pyridine silver preparation (15–1–4). × 1450.

6 and 7 Bipolar neurons of the hypoglossal nerve in the ventral wall of the inferior olivary nucleus of a 5-month-old human fetus (no. 70). Both figures are from the same section (15–3–4). In figure 6 a nerve cell is shown at the edge of the bundle of hypoglossal nerve fibers. Other neurons are present in the field, but are out of focus. Pyridine silver preparation. × 1280.



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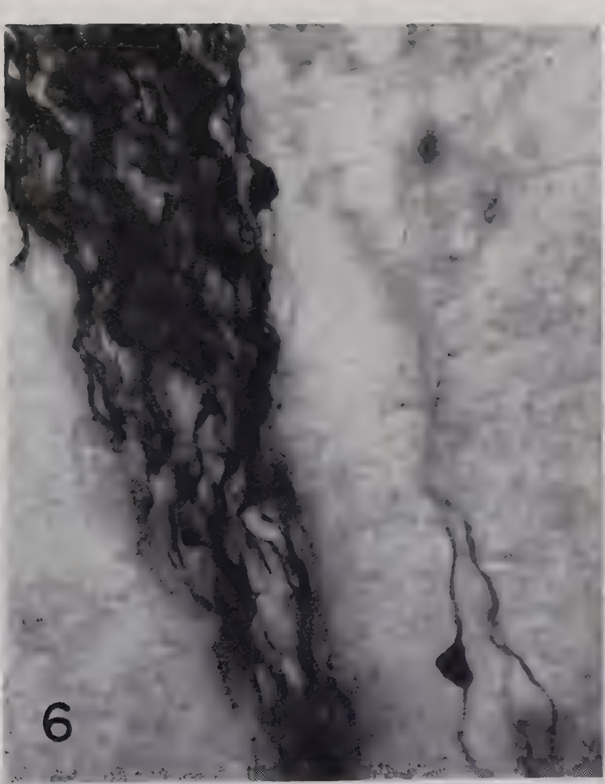

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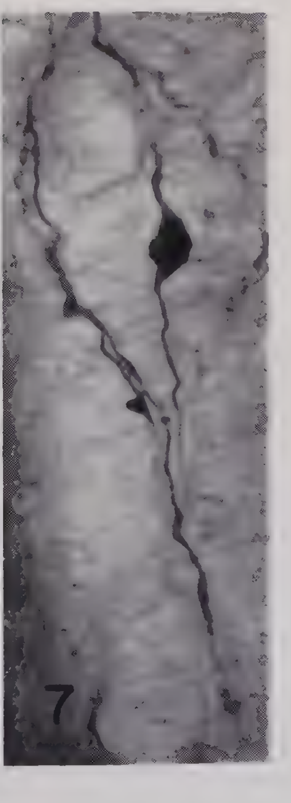

7

FACTORS INFLUENCING DEVELOPMENT OF SCROTUM

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ONE PLATE (FIVE FIGURES)

It is generally known that the scrotum plays an important physiological role inasmuch as it helps to regulate testis temperature which in turn influences spermatogenesis. Yet much less attention has been given to the hormonal regulation of the scrotum than to that of the other accessory sex organs. It has been established that in the immature rodent (Steinach and Kun, '40; Hamilton, '36) or monkey (Hamilton, '38) as well as in cryptorchid human beings (McCullagh and McGurl, '39; Kearns, '39) treatment with testosterone and other testoid compounds causes a descent of the testis into the scrotum with a corresponding increase in the size of the latter. However, these observations cannot answer the question whether the testoids act on the scrotum directly, or through the intermediary of the testis which distends the scrotum by its mere descent. The studies of Phillips ('38), Phillips and Andrews ('36), Andrews ('40) and Tyrrell, Andrews and Zelle ('42) led them to conclude that the mere mechanical presence of the testis plays no part in maintaining the scrotum in a physiologically normal condition and that the internal secretion of the gonad is the sole regulator of scrotal activity. They arrived at this conclusion by physiological experiments indicating that after castration the contractility of the tunica dartos muscles is severely damaged and cannot be maintained by substituting steel balls for the gonads.

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Although these experiments convincingly show that the mere presence of a heavy foreign body does not suffice to cause normal scrotal development, they do not prove that mechanical factors are not involved in the development of this organ. From numerous observations made in this laboratory on castrate rats treated with large doses of various testoid compounds the impression was gained that even if the treatment caused the seminal vesicles and prostates to undergo enormous development it never resulted in the formation of a normal scrotum. In the writer's opinion the experiments described in this communication show that both hormonal and mechanical factors play a role in scrotal development.

EXPERIMENTAL

In the first experiment twenty immature albino rats weighing 26–40 gm. (average 33 gm.) were castrated through a suprapubic incision after placing a single ligature around the blood vessels and vasa deferentia of both testes. The epididymis was removed with the gonad. These technical details are emphasized because if a castration is performed in this manner scar tissue develops, forming a bridge between the two spermatic cords, which actually pulls on the gubernaculum testis and hence counteracts any possible pressure that the intra-abdominal organs may exert on the inguinal canal and the region from which the scrotum will subsequently be formed. It should also be pointed out that at the time of castration the testes of these animals had not descended and hence a scrotum was not developed as yet. Immediately after castration the animals were divided into two groups of ten. The first group remained untreated while the second group received bidaily subcutaneous injections of 500 γ of testosterone in 0.1 cc. of peanut oil. One month after initiation of the treatment all animals were killed. It was found that at this time neither the castrate controls nor the testosterone treated castrates showed any sign of scrotum development, although the seminal vesicles of the treated animals averaged 500 mg. (range 412–552 mg.) and the prostates 296 mg. (range

244–406 mg.) as compared with an average weight of 13 mg. (range 11–16 mg.) for the seminal vesicles and 19 mg. (range 13–29 mg.) for the prostates of the untreated controls. It may be said that the seminal vesicles and prostates of the treated castrates were actually larger than those of intact males of the same age, yet their scrotum remained undeveloped, while that of normal males of the same age and weight is invariably fully formed (fig. 1).

Thus it appears that in the absence of testicular tissue a disproportion exists between the ability of testosterone to stimulate the seminal vesicles and prostates and its inability to cause scrotal development. It was hence decided to investigate whether the hormone could prevent the scrotal atrophy which normally occurs after castration in post-pubertal rats. Twenty albino rats weighing 115–140 gm. (average 127 gm.) were castrated using the same technic as in the previous experiment.

Immediately after castration the animals were subdivided into two groups of ten. The first group remained untreated while the second group received bidaily subcutaneous injections of 2.5 mg. of testosterone propionate in 0.1 cc. of peanut oil. In this series the more active propionate was used and given in a considerably higher dose than was employed in the first experiment because the animals were correspondingly larger. Treatment was continued for 75 days. At the end of this period the scrotum of both groups was significantly smaller than that of intact normal males of the same age in our colony. However, the involution of the scrotum was less pronounced in the treated than in the untreated castrates. The seminal vesicles of the treated animals averaged 1387 mg. (range 1116–1746 mg.) and the prostates 906 mg. (range 665–1143 mg.) as compared with an average weight of 39 mg. (range 20–61 mg.) for the seminal vesicles and 31 mg. (range 26–36 mg.) for the prostates of the untreated controls. The seminal vesicles and prostates of our treated castrates were considerably larger than those of intact males of the same age. Yet some involution of the scrotum undoubtedly occurred as a result of castration. Hence it appears that even excessive doses of testoid compounds cannot completely prevent scrotal involution following gonadectomy (fig. 2).

A third experiment was then conducted on thirty very young albino males weighing 21–28 gm. (average 25 gm.). Ten of these animals were castrated after placing a separate ligature around the pedicle of each testis so as to prevent any traction on the gubernaculum. In these the epididymis was removed together with the testis. A second group of ten animals was gonadectomized by severing the communication between testis and epididymis leaving the latter intact. The remaining ten animals were similarly castrated but an "artificial testis" was substituted for the gonad. Such artificial testes were made from glass tubing which had a diameter of 7 mm. and was cut into pieces of 1.7 cm. in length. The sharp edges of the tubing were rounded off in the flame of a Bunsen burner and a silk thread was led through the lumen. With aid of this thread the beads were sewn on to the head of the epididymis on one side and to the tail of the organ on the other side. In this manner they occupied the natural position of the testis which of course at this time was still above the inguinal canal. Immediately after these various operations all thirty animals were treated with bidaily subcutaneous injections of 500 γ of testosterone propionate in 0.1 cc. of peanut oil for 1 month. At the end of this period scrotum development was very slight in the animals of the first group (testis and epididymis removed) but practically normal in those of the second (only testis removed) and third (testis substituted by glass bead) groups. It is believed that the moderate degree of scrotal development which occurred in the first group of this experiment was due to the fact that in this case, the pedicles of the testes were separately ligated so that some pressure could be exerted by the intraperitoneal organs on the inguinal canal. Yet the presence of an epididymis or a glass bead in the scrotum resulted in an even more pronounced enhancement of the scrotum-forming action of testosterone (figs. 3, 4, and 5).

It may be said that an additional experiment in which 10 mg. of testosterone propionate were administered daily to immature castrate rats similar to those of group 1 in the above series, also failed to cause complete scrotum formation within 1 month, although this enormous dose enlarged the seminal vesicles and prostates to what might be termed gigantic proportions.

On the basis of the experiments reported in this communication it appears justified to conclude that both the mechanical distention of the scrotum and the stimulating effect of testoid compounds are required for the development of a normal scrotal pouch. Such a synergism between hormonal and mechanical factors has repeatedly been seen to play a role in the induction of morphological changes in accessory sex organs. Thus Selye, Borduas and Masson ('42) found that the musculature of the uterine wall exhibits much more pronounced development in progesterone treated rats if the lumen of the uterus is mechanically distended than if it is collapsed. Similarly Selye and Clarke ('42) demonstrated that while under normal conditions progesterone causes mucification of the vaginal epithelium in spayed rats it elicits cornification of the surface lining if the vaginal lumen is subjected to mechanical distention.

SUMMARY AND CONCLUSIONS

Experiments on castrate albino rats indicate that testosterone in itself does not induce full scrotal development in immature rats nor does it completely inhibit scrotal involution in gonadectomized adults unless the scrotum is mechanically distended. Distention by the epididymis or an ''artificial glass testis'' can sensitize the scrotum to the action of testosterone. Without this mechanical stimulus the scrota of castrate rats remain subnormal even though the animals receive testosterone in doses sufficient to develop the seminal vesicles and prostates far beyond their normal size.

ACKNOWLEDGMENTS

The expenses of this investigation were defrayed through the Blanche E. Hutchinson Fund of McGill University. The author is greatly indebted to Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J. who supplied the testosterone and testosterone propionate necessary for these studies.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

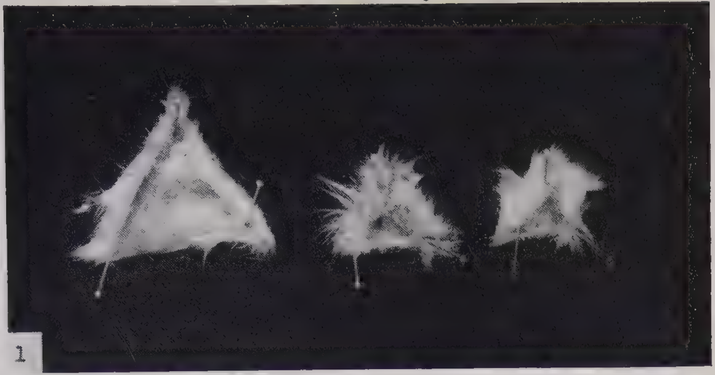
1 (Reading from left to right.) Dissected scrotum of intact male, castrate male treated with testosterone and untreated castrate male. Note that in these prepubertally castrated rats testosterone failed to cause scrotal development in the absence of the gonad.

2 (Reading from left to right.) Dissected scrotum of intact male, castrate male treated with testosterone and untreated castrate male. Note that in postpubertal castrates the involution of the scrotum is somewhat inhibited but not prevented by testosterone propionate.

3 Scrotal region of prepubertally castrated rat treated with testosterone propionate. Note very slight degree of scrotal development.

4 Treatment as in the rat shown in figure 3 but epididymis not removed at time of castration. Note that due to the presence of this organ testosterone caused almost normal scrotal development.

5 Treatment as in the rat shown in figure 4 but glass beads substituted for testes. Note that this additional mechanical distention sensitized the scrotum to testosterone sufficiently to make normal scrotum development possible.



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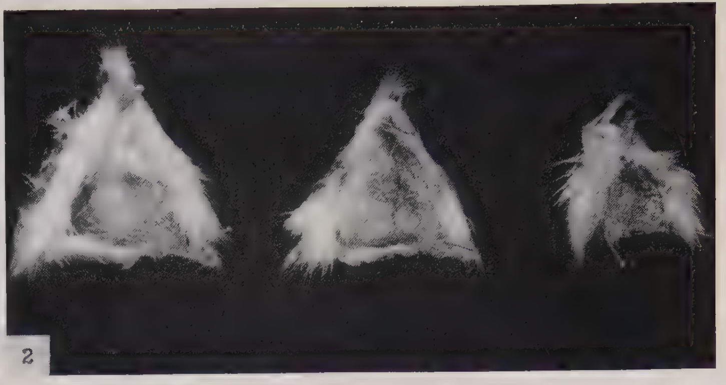

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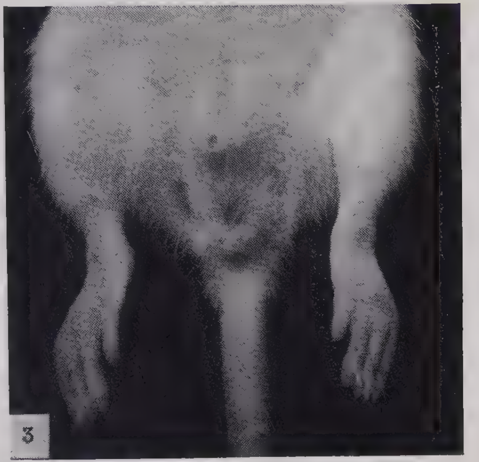

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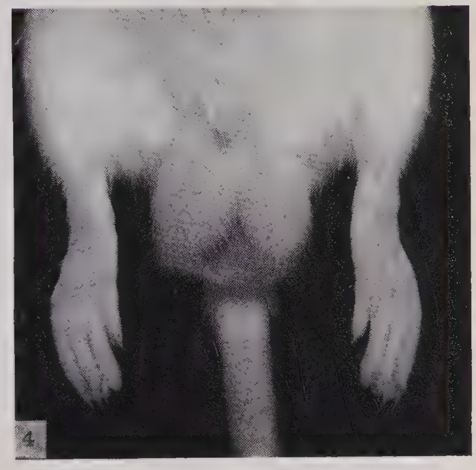

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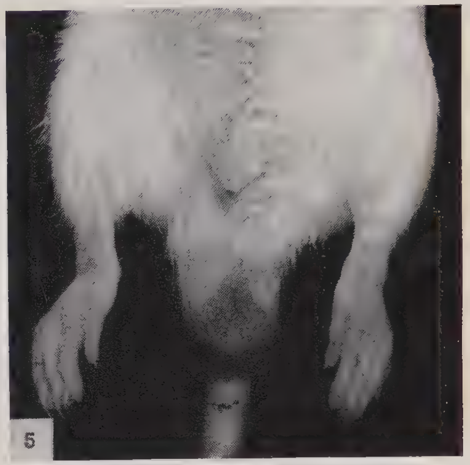

5

SEXUAL DEVELOPMENT IN SMALL AND LARGE TYPES OF SWINE

RALPH W. PHILLIPS AND JOHN H. ZELLER
United States Department of Agriculture, Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.

SEVEN FIGURES

INTRODUCTION

Considerable variation in conformation exists in each of the species of domestic animals as a result of selection practiced by man in an effort to fill his various needs. Marked differences in conformation exist even within supposedly pure breeds. A striking example of such variation is found in the Poland China breed of swine; three types, characterized as small, medium and large have been studied at the Beltsville Research Center, Beltsville, Maryland since 1931. Results of studies of these animals which bring out differences in their conformation and chemical composition have been reported by Hankins ('34), Hankins and Ellis ('35), Zeller ('40) and Hankins ('40).

The types differ in reproductive capacity as well as in conformation. More difficulty has been experienced in maintaining normal reproduction in the small than in the larger types, and libido has seemed less pronounced in the small type. Hetzer and Brier ('40) found highly significant differences between the reproductive levels of the three types as measured by size of litter. Average litter size in the large type was 7.4 as compared to 5.8 in the small type. Medium type sows had litters of intermediate size. The study herein reported was undertaken to determine some of the basic reasons for the apparent differences in reproduction, using the two extremes in type.

MATERIAL AND METHODS

Small and large types of Poland China swine were used in this study. The small type animals were short, thick-bodied, and low set. The large type animals had longer bodies and legs and were not so thick-set or fat. However, they were not of the extreme rangy type found in some sections of the country. Differences in type become apparent at an early age, as may be seen from the typical examples of the two types at various ages shown in figure 1.

In a study of testicular development, forty-four large and thirty-five small type boars were used. These animals were

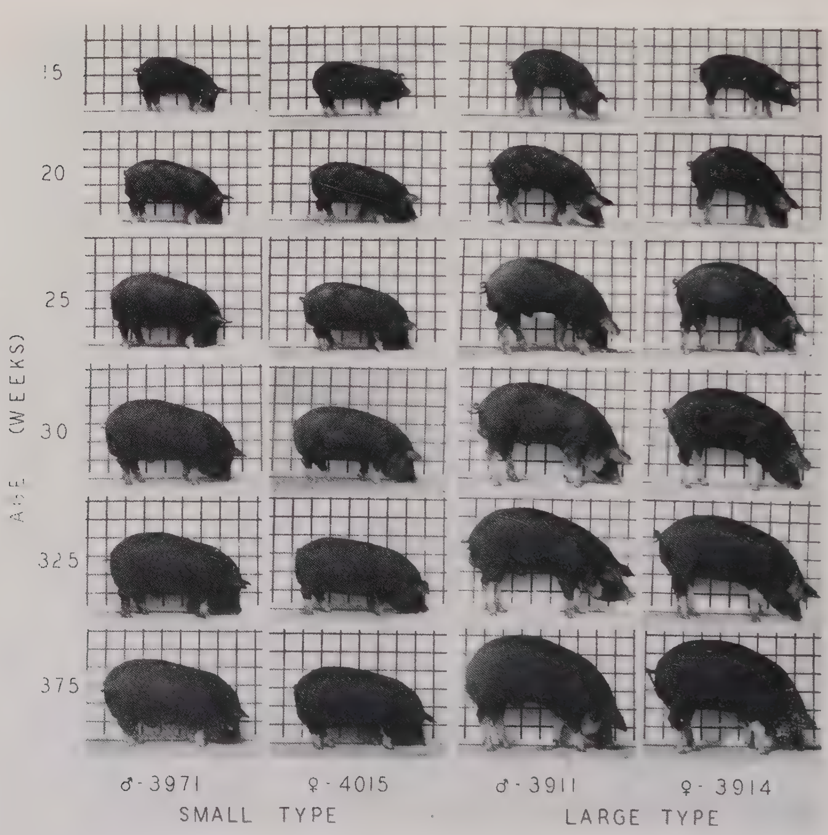

Fig. 1 Typical small and large type Poland China hogs. Each individual is shown at six different ages.

castrated at ages varying from 5 to 40 weeks and data were obtained on body weight (before castration) and weight of testes (with epididymis removed). Blocks of tissue were excised from a standard location in the left testis, fixed, imbedded, sectioned, and stained with hemotoxylin and eosin for study of the presence of spermatozoa and size of the seminiferous tubules.

Twenty-four small and thirty-nine large type gilts were observed daily for estrus from a time shortly after weaning until the first estrous period was noted. Visual observations were made for swelling of the vulva and ''teaser'' boars were used to determine the actual occurrence of estrus.

DATA AND DISCUSSION

Sexual development in the male

The presence of spermatozoa in the seminiferous tubules is commonly accepted as a measure of sexual maturity. In this study 100 tubules, selected at random, were observed for the presence or absence of spermatozoa in the sections of tissue from each boar. These data are presented in graphic form in figure 2. No spermatozoa were observed in the tubules prior to 20 weeks, and in some cases in both types none were found at 20 and 22.5 weeks. Spermatozoa were found in some tubules in all individuals 25 weeks of age or older. Careful inspection of figure 2 indicates that boars of both types develop spermatozoa at about the same average age, but that in small type boars there is greater variability in the time when a major portion of the tubules begin producing spermatozoa. The findings on age at which spermatozoa first appear in the testes are in general agreement with earlier work by Phillips and Andrews ('36), in which spermatozoa were first observed in the testes of Chester White and Berkshire boars at 21 weeks. Up to 17.5–20 weeks the tubules, as seen in cross section, have a layer of spermatogonia around the basement membrane and the remainder of the tubule is filled with an opaque mass. Occasional cells appear in this material and become more frequent as the time of sexual maturity approaches. However, the organization of the epithelium into the form commonly seen in the mature animal comes about after 17.5 weeks. After this age the lumen of the tubule appears and proliferation of the germinal epithelium is apparently quite rapid since spermatozoa were found by 20 weeks in some cases and in all cases after 22.5 weeks.

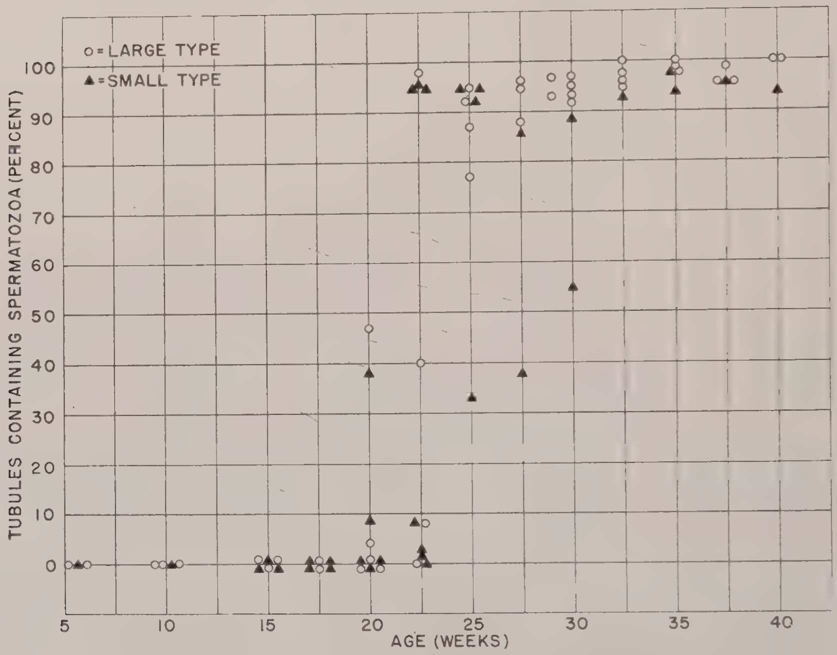

Fig. 2 Showing the proportion of seminiferous tubules containing spermatozoa at various ages. One hundred tubules were observed in each case.

The changes in diameter of seminiferous tubules are shown in figure 3. The tubules apparently undergo little, if any, change in size between 5 and 10 weeks. Rapid growth begins some time between 10 and 15 weeks and continues until about 27.5–30 weeks. Inspection of figure 3 does not reveal any marked difference in rate of development of the two types up to about 27.5 weeks, but there is a tendency for tubules to be larger in the large type boars after this age, indicating the possibility that mature size of tubules may be greater in the large than in the small type. Observations on a considerable number of mature boars would be necessary to decide this point.

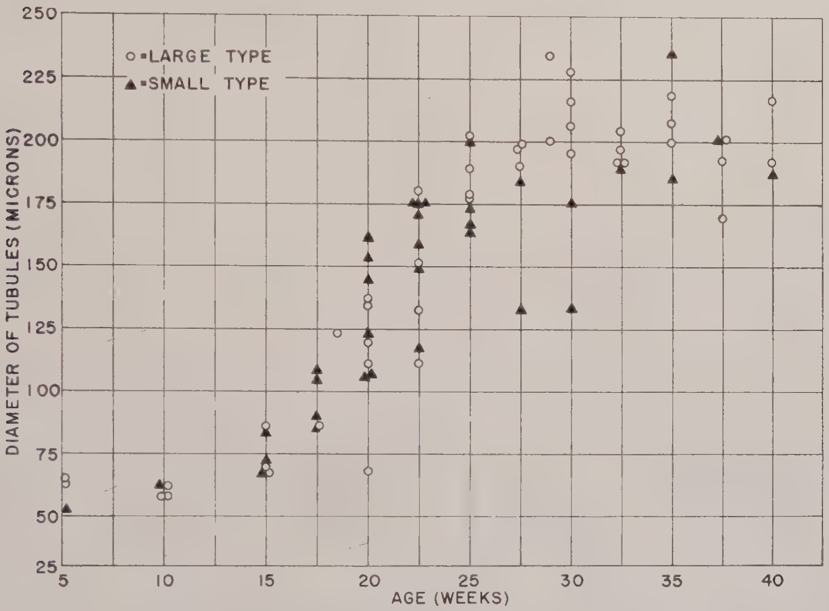

Fig. 3 Showing the diameter of seminiferous tubules at various ages. Fifty tubules were measured in each case.

Changes in the weight of the testes with age are shown in figure 4. There is a very slight increase from 5 to 10 weeks, and a somewhat greater increase from 10 to 15–17.5 weeks. Beyond this age there is a rapid increase in the weight of the testes. This rapid increase in weight apparently begins a few weeks after the tubules begin to increase rapidly in diameter. Inspection of figure 4 does not reveal any diferences between small and large type boars through 25 weeks of age. Beyond this age the results are difficult to interpret. One small type boar had the largest pair of testes observed; these weighed 632.4 gm. at 35 weeks. With this one exception the testes of small type boars tend to be lighter than those of large type boars at all ages above 25 weeks. Thus, there appears to be a tendency for the testes of small type boars to develop less rapidly in weight than those of large type boars, after sexual maturity is reached. Greater numbers in each of the age groups studied and in age groups beyond 40 weeks would be necessary before a definite conclusion could be reached on this point. Average weights of the testes at the various ages are given in table 1.

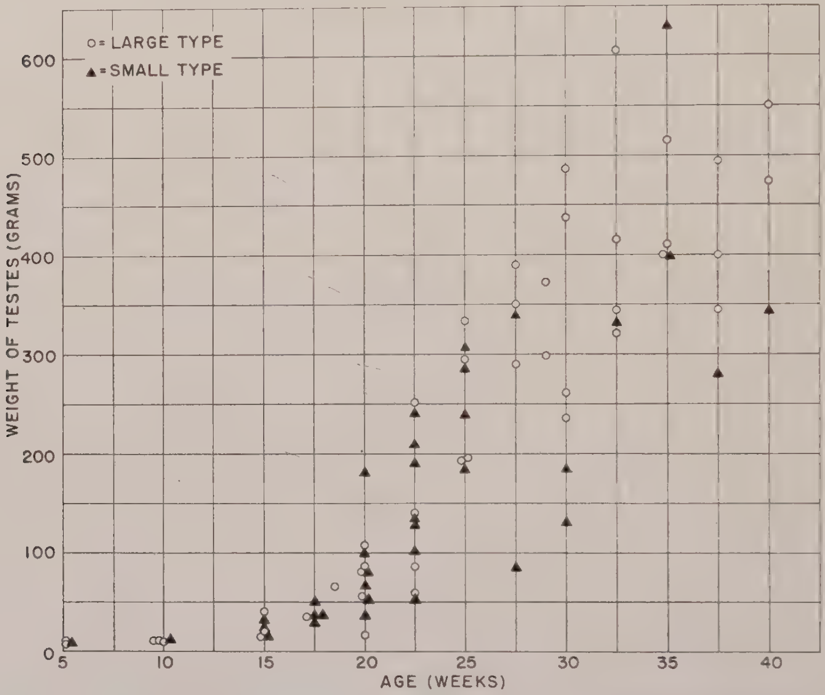

Fig. 4 Showing changes in weight of testes with age. Combined weight of right and left testes is used in each case.

Weight of the testes was also studied in relation to body weight, and the data are shown graphically in figure 5. In both types, the testes increased gradually in size as body weight increased up to 90 pounds. Beyond this point the testes increased at a relatively more rapid rate than did body weight. Considerable variability exists in both types and there is no definite indication of a difference in the rate of development.

TABLE 1

Average weights of left and right testes at various ages in small and large type boars.

AGE	SMALL TYPE			LARGE TYPE		
	No.	Left testis	Right testis	No.	Left testis	Right testis
weeks		*gm.*	*gm.*		*gm.*	*gm.*
5	2	5.0	4.5	1	4.0	3.5
10	3	6.0	5.8	1	6.0	6.0
15	3	12.9	12.8	3	12.6	11.7
17.5	1	18.0	17.0	4	19.4	18.6
18.5	1	34.2	31.6	..	...	...
20	5	35.9	35.4	6	44.1	41.5
22.5	4	66.3	66.6	7	77.3	75.4
25	4	130.2	124.2	4	135.4	120.7
27.5	3	171.8	170.0	2	113.3	100.4
29	2	179.8	156.2	..	...	...
30	4	183.5	172.0	2	80.4	78.0
32.5	4	219.3	202.5	1	167.3	165.6
35	3	224.2	217.3	2	267.2	248.4
37.5	3	211.8	201.9	1	144.5	137.0
40	2	246.8	264.6	1	172.8	172.3

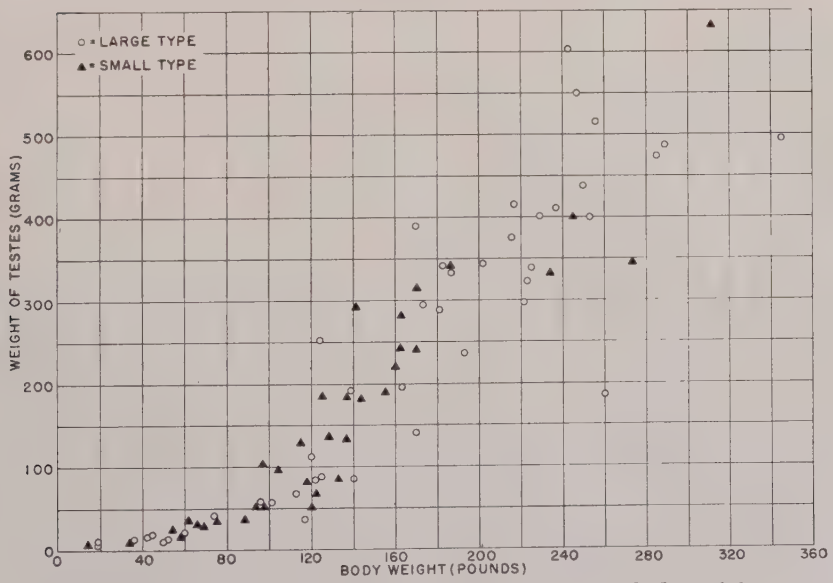

Fig. 5 Showing the relation of weight of testes to body weight.

Increase in the weight of the testes was also studied by determining the percentage that the weight of the testes was of the total body weight. These figures are plotted against age in figure 6. The relative size of the testes actually decreased from 5 to 10 weeks. This is true because the testes increased in weight only slightly during this period (fig. 4) while the pigs gained considerable in body weight. The first marked increase in the relative size of the testes was observed at 20 weeks, or at the time when spermatozoa were first found in some individuals. No marked difference appeared between the rate of development of the two types, but beyond 25 weeks of age a few large type boars were above the level of the major portion of the boars and a few small type boars were below this level, indicating the possibility of slightly larger testes in relation to total size in the large type.

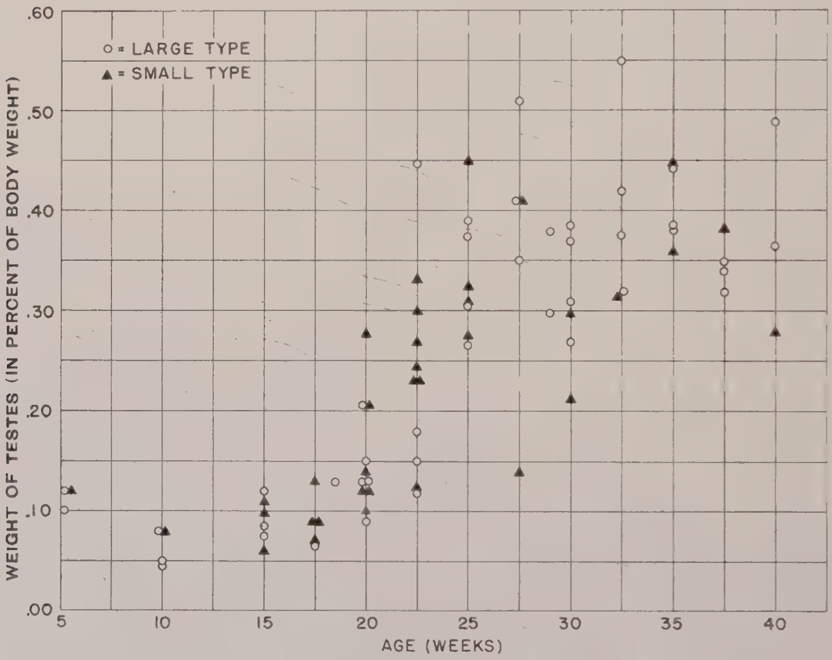

Fig. 6 Showing changes in weight of testes in proportion to body weight with changes in age.

The data include some information on the relative size of the left and right testes that is of interest. In thirty-one of the forty-four large type boars the left testis was larger and in one case the weights were equal. In the group of thirty-five small type boars the left testis was larger in thirty, the right was larger in four, and in one boar the weights were equal. The average weights of the left and right testes in each age group in each type are shown in table 1. The average age of all the large type boars listed is 25 weeks and the average weights of left and right testis are 122.1 and 117.3 gm., respectively. In the small type the average age is 22.9 weeks and the average weights of left and right testes are 82.3 and 77.3 respectively.

Sexual development in the female

Wide variations were observed in the age at first estrus in both types. The earliest initial estrus periods occurred at 160 and 159 days and the latest at 259 and 249 days, respectively in the small and large types. The distribution of ages at first estrus for the two types are shown in figure 7. The distribution for the large type approximates a normal curve but that for the small type is quite different. One point of particular interest in the small type group is that the five gilts that came in estrus between 160 and 169 days (fig. 7) were litter mates and all actually came into estrus between 160 and 165 days.

The average age at first estrus was 207.8 days in the small type and 198.7 in the large type. By the use of an analysis of variance it was determined that these two means do not differ significantly from each other. The F value obtained was 2.05, and an F of 4.00 was necessary for significance at the 5% level.

The average weight at first estrus was 189.5 pounds in the small type and 199.3 in the large type gilts. These means did not differ significantly from each other, the F value being 1.11, as compared with a required value of 4.00 for significance at the 5% level.

Even though the differences in age and weight at first estrus were not statistically significant, they indicate a tendency for more rapid development in the large type, since females of this type not only came into first estrus approximately 9 days earlier, on the average, but at this earlier age weighed about 10 pounds more than did the small type at the age they first came in estrus. Since it is not only important to have sows come into estrus at an early age but also that they should be large enough to breed, it seemed worthwhile to analyze the data for age at first estrus, making appropriate adjustments for differences in weight. This was accomplished by the use of an analysis of covariance. With the average weight at first estrus held constant (at 194.4 pounds, which is an average of the means of the two types) the ages at first estrus were 211.5 and 195.1 respectively in the small and large types. These

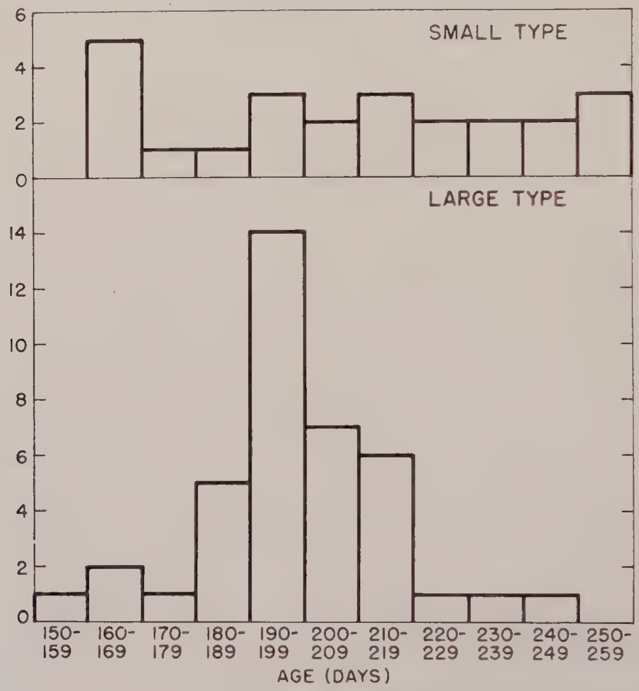

Fig. 7 Variations in age at first estrus in small and large type gilts.

means differ significantly from each other, the F value being 5.05, as compared with required values of 4.00 and 7.08 respectively for the 5 and 1 per cent levels of significance.

A comparison of the average weights and ages at first estrus in the two types indicates that there is a negative correlation between age and weight at first estrus. Since this correlation is based on only two points, the means of the two types, it is — 1.0. This relationship does not hold within the types, however, for the coefficient of correlation between weight and age at first estrus is + 0.51 in this case and this relationship is highly significant. The regression of weight on age within types is + 0.75, indicating that for each day's change in average age at first estrus there is a corresponding change in weight of 0.75 pound.

DISCUSSION

The findings reported in this paper indicate that males of both the large and small types reach sexual maturity at essentially the same time but there is an indication that the development of full spermatogenic activity may be delayed in some small type boars and that the size of the testes and seminiferous tubules at maturity may possibly be greater in the large type. The age at first estrus is less in the large than in the small type and the difference between the two types is significant when weight is held constant.

Earlier work (Hetzer and Brier, '40) shows that large type sows have larger litters than small type sows. Observation of the herds over a period of years indicates that the large type show more pronounced libido. Maintenance of a satisfactory level of reproduction in the small type herd has been difficult.

In general, the data and observations indicate a higher level of sexual activity in the large type, and a more pronounced difference between types in females than in males. It is possible that this difference in the sexes reflects a difference in the sensitivity of the sexes to gonadotropic hormones. The difference in size of skeleton between the two types, and the greater litter size in the large type suggest a higher level of pituitary activity in this type. The pituitary gland exerts a vital role in the maintenance of spermatogenetic activity and precocious production of spermatozoa has been brought about in a number of species in seasonal breeding types by administering pituitary substances or other substances having somewhat similar effects (Moore, '39), but attempts to stimulate precocious activity in species that produce spermatozoa throughout the year have met with relatively little success. Precocious development in the female has been brought about in several species of the year-round breeding type (Fevold, '39; Zondek, et al., '42). Therefore, it seems possible that there may be a higher level of pituitary activity in the large type and the extra stimulation is sufficient to cause somewhat earlier sexual development in the female, while not causing any measurable differences in the age of sexual maturity in the male, as measured by the age at which spermatozoa are first produced.

SUMMARY

Sexual development in swine was studied, using small and large type animals of the Poland China breed. The animals studied varied in age from 5 to 40 weeks. Thirty-five small and forty-four large type boars, and twenty-four small and thirty-nine large type gilts were used.

Spermatozoa were first observed in the seminiferous tubules at 20 weeks and were present in all boars observed at 25 weeks and later ages. Rapid development of the germinal epithelium began after approximately 17.5 weeks of age.

The seminiferous tubules began to increase in size between 10 and 15 weeks and continue to grow rapidly up to about 27.5–30 weeks. After this age there appeared to be little increase in size.

The testes appeared to increase slightly in weight between 10 and 17.5 weeks, and began to grow rapidly between 17.5 and 20 weeks. Growth appeared to continue at a fairly constant rate from this point up to 40 weeks. In relation to body weight, the testes increased slowly in size up to a body weight of 80–85 pounds. Beyond this point the increase in weight of

testes is more rapid and it continued at a fairly constant rate up to the maximum weights used in this study.

The testes decreased somewhat in size in proportion to body weight between 5 and 10 weeks. The relative size of the testes increased slightly between 10 and 17.5 weeks and at a more rapid rate from 17.5 to about 30–32.5 weeks. Beyond this point data indicate that the relative size may become stabilized at about 0.4% of total body weight.

No clear cut differences appeared in the rate of sexual development of males of the two types. The testes of the small type produced spermatozoa at the same age as those of the large type. However, there is some evidence that the development of the testes from this point may be more variable in the small type and that the final development of the testes may not be as great, as judged by actual and relative weight and by diameter of seminiferous tubules.

The left testis was heavier than the right in most cases.

Gilts of the small type showed first estrus at an average age of 207.8 days and an average weight of 189.5 pounds. For the large type the averages were 198.7 and 199.3, respectively. Differences between the two types in average age and weight were not statistically significant. When adjusted to a common weight level, the large type showed first estrus at 195.1 days as compared to 211.5 in the small type and these means differ significantly.

Correlation between weight and age at first estrus was + 0.51 within types, as contrasted to a negative relationship between the two types.

The distributions of ages at first estrus differed materially in the two types. In the large type the distribution approximated a normal curve, while in the small type it was quite flat and irregular, indicating that the age at first estrus was not so well fixed in the small type stock used in this study.

The findings reported, and observations based on breeding experience with the two types indicate a lower level of sexual activity in the small type and the possibility of a lower level of pituitary gland activity in the small type is suggested.

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THE EFFECT OF REMOVAL OF A LARGE PART OF THE LYMPHOID SYSTEM ON THE WEIGHT OF THE PORTION REMAINING IN SITU [1]

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The extent and distribution of hypertrophy in the lymphoid system following removal of portions of this system has been the subject of a number of investigations. Since the work of Rouvière and Valette ('37), who review the earlier literature, it appears to be the concensus of opinion that following complete removal of one lymph node, no new formation occurs at its site. Partial removal may be followed by an increase in size of the fragment remaining. In such experiments the effect, if any, on the other lymphoid structures was not studied.

Whitney ('28) in reviewing the effects of splenectomy states, "There is a slight initial enlargement of the lymph nodes but it does not persist. There is little if any increase in the lymphoid elements in the bone marrow." In discussing the work of Wiseman ('31), Drinker and Yoffey ('41) suggest ". . . the other lymphoid tissues are well able to compensate for the absence of the spleen, a finding which accords with many other observations on the relationship between splenectomy and the remainder of the lymphoid organ." Bracco ('39) found that after splenectomy in the rabbit there was a hyperplasia of the follicles and of the reticular endothelial elements in the lymph nodes. Jordan and Robeson ('42) observed in the pigeon that following sub-total splenectomy there was an increase of lymphoid activity in the bone marrow and the remaining portion of the spleen, and that after total splenectomy there was an increase in lymphoid tissue in the bone marrow.

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[2] With the technical assistance of Tohru Inouye.

The most nearly complete extirpation of the organized lymphoid tissue has been carried out by Sanders and Florey ('40) on rats infected with Bartonella. These investigators found that there was no new formation of lymph nodes, but that there were "masses of newly-formed lymphoid tissue along the branches of the portal system and hypertrophy of the peribronchial lymphatic tissue." In rabbits similar but less distinct changes were observed by these investigators.

In their summary of the problem, Drinker and Yoffey ('41) conclude that "there is the suggestion of some compensatory activity on the part of the remainder of the lymphoid tissue following the removal of parts of the system" but that there is "no precise information concerning the lymphoid tissues as a whole" following such extirpation.

Studies here reported were designed to demonstrate what lymphoid node weight changes follow extensive removal of the system. In view of the fact that no precise work on the lymphoid node weights in mice is available, the studies reported here were designed to demonstrate what lymph node weight changes could be observed with extensive removal of a large proportion of the lymphoid system.

MATERIAL AND METHODS

Experiments were carried out on mature male albino mice which were young enough to include the period of rapid growth during the experimental period. Preliminary studies were performed to determine the minimum age at which removal of large proportions of the lymphoid system could be carried out with survival. When the extirpations were carried out on animals of 55 days of age, 10% survived; at 100 days of age, 20% survived; and at 120 days, 75% survived. In view of these results we used animals 122 days of age at the beginning of the experiment.

Operations were conducted under ether anesthesia in three stages with an interval of 3 days between each stage. In stage I the superficial cervical and inguinal nodes were removed, in stage II the axillary nodes, and in stage III the mesenteric mass and the spleen. Ten days following the last stage, the animals were killed with ether, placed in a Sperry and Brand ('39) moist box at 37°C., the remaining or "test" nodes excised and weighed to the nearest 0.1 mg. on a torsion balance.

In those animals which died during or after the second operation a necropsy was performed immediately after death, and the weights of the nodes determined as above. This group is called the "short survival group."

Another group of animals was subjected to the same 3-stage operative procedure as that described for the operated group, except that the nodes were exposed, but not removed. This group is designated as the "sham operated control group."

Finally a control group, not subjected to any operation was necropsied in the same manner.

The following tabulation shows the proportion of the lymphoid system which we succeeded in removing in our operations. The figures given are mean weights in milligrams of lymphoid tissue in the thirteen normal control animals (unoperated control group). The nodes designated as "removed" are comparable to those nodes which were actually removed in the groups undergoing operations; those designated as "remaining" are comparable to those which remained in situ until the time of autopsy in the operated control group.

Lymphoid tissue "removed"	
Lymphoid nodes (superficial cervical, inguinal, axillary, and mesenteric)	44.32 mg.
Spleen	72.40 mg.
Total "removed"	116.72 mg.
Lymphoid tissue "remaining":	
Lymph nodes (popliteal, deep cervical, iliac, and retroperitoneal	13.85 mg.
Thymus	30.72 mg.
Total "remaining"	44.57 mg.
Total lymphoid tissue of the mouse:	
Lymph nodes and thymus (excluding spleen)	88.89 mg.
Lymph nodes, thymus and spleen	161.29 mg.
Percentage of lymphoid tissue "removed"	
Excluding spleen	49.86%
Including spleen	72.37%

It is admitted that the percentages of removal are in error to some extent due to the fact that the unorganized lymphoid masses such as those in the mucosa of the intestine and a few small scattered nodes do not appear in the calculations.

RESULTS

The response of the lymphoid system to a removal of a large part of it was determined by a study of the weight changes of the nodes left in situ: the popliteal, deep cervical, iliac, and retroperitoneal nodes. These are designated as the "test" nodes. The mean value for each group of test nodes and for the weight of all nodes is given in table 1. Throughout the paper the significance of difference between means is stated in terms of P, the probability that such a difference would occur by chance, i.e., a P of 0.02 means that there are

TABLE 1

Effect of removal of lymphoid tissue in the mouse.

	UNOPERATED CONTROLS	SHAM OPERATED CONTROL GROUP	SHORT SURVIVAL GROUP	OPERATED GROUP
No. of mice	13	13	13	15
Mean age in days	122	122	122	122
Mean body wt. in grams	20.2	21.7	21.4	21.0
Total wt. in mg. of "removed" nodes	44.32 ± 9.85	48.42 ± 16.55	40.01 ± 8.45	39.51 ± 16.7
Wt. of "test" nodes in mg.				
Popliteal	4.71 ± 3.02	3.00 ± 2.05	3.98 ± 1.93	4.27 ± 2.00
Deep cervical	1.05 ± 0.56	1.48 ± 1.23	1.67 ± 1.27	1.64 ± 1.18
Iliac	3.59 ± 1.01	3.47 ± 3.06	3.94 ± 2.48	4.75 ± 3.34
Retroperitoneal	4.51 ± 0.29	3.17 ± 2.05	1.68 ± 1.40	6.85 ± 4.53
Total wt. in mg. of "test" nodes	13.85 ± 4.27	11.12 ± 6.75	11.26 ± 4.08	17.51 ± 6.04
Wt. of thymus in mg.	30.72 ± 9.79	32.93 ± 8.19	30.58 ± 7.95	30.46 ± 10.10

two chances in one hundred of its occurring in random sampling. The formula used was that of Simpson and Roe ('39) and the tables those of Fisher and Yates ('38).

The non-specific effect of the operative procedure may be ascertained by a comparison of the unoperate group with the sham operate group. The mean weight of the test nodes of the sham operate group was somewhat below that of the unoperate control. This difference is one of questionable significance ($P = 0.2$). In addition, however, the same general tendency appears from the fact that the mean weight of "removed nodes" in both the short survival and operate group is smaller than that of the corresponding nodes in the unoperated controls. The statistical significance, however, is negligible (P values are 0.5 and 0.65 respectively). However, the thymus does not participate in this change, the mean weight in the sham operates being if anything higher than in the control. Selye ('37) has demonstrated that a wide variety of injurious agents can elicit what he terms an "alarm reaction," one feature of which is a decrease in size of the lymph nodes and thymus. Since this fails to occur after adrenalectomy, and since a similar involution occurs with the administration of adrenal cortical hormone, Selye suggests that it may be due to hypersecretion of the adrenal cortex. It is possible that the changes produced in our "test" nodes by non-specific operative procedures are a manifestation of this reaction. Selye's hypothesis is not supported by our data on the thymus.

Since the mean weight of the test nodes in the operate group is higher than that of the unoperated controls ($P = 0.08$), the removal of a large portion of the lymphoid system has increased the weight of the remaining nodes. Further, if account is taken of the decrease in size of the test nodes which occurs in the sham operates at a comparable time after operation the magnitude of the effect of the removal is even more apparent and amounts to 46% of their original weight. The statistical significance of this increase is high ($P = 0.015$). The effect of the operation has been studied by an analysis of variance. The variance between the control, sham operate and short survival groups did not differ significantly from the variance within these groups, a fact which indicates that these three groups were homogeneous. However, when the operate group was included in the series, the variance analysis showed that the groups were now heterogeneous. This increase was found to have occurred 16 days after the first stage of the operations or 10 days after the third stage had been completed. However, no such increase was seen in animals dying shortly after the third stage, "short survival group." Our data do not permit us to define the time relations more precisely. It is possible then, if our necropsies had been performed at a time other than 10 days after the last stage, that an even greater weight gain might have been found. The operations did not affect the weight of the thymus.

Distribution of weight increase. In order to determine whether the increase occurred in all the test nodes or was confined to certain regions, the data for the groups of test nodes was analyzed. The mean values for these also appear in table 1.

The operative procedures apparently had no demonstrable effect on the popliteal, deep cervical, and iliac nodes, since the differences in the mean weights of these nodes in the various groups of animals were not significant ($P = 0.1$ to 0.9). However, significant changes are found in the retroperitoneal nodes. There is a decrease in the same operates as compared with the unoperate controls ($P = 0.03$) which may be regarded as a manifestation of the "non-specific" effect of operation. In the operate group, moreover, there is an increase over both the unoperate ($P = 0.06$) and the sham operate ($P = 0.01$) groups. The increase in weight of the retroperitoneal nodes is 82% of their original weight.

From these data it appears that the influence of removal of a large proportion of the lymphoid system is not a general one affecting all the test nodes uniformly, but is to a considerable extent localized in the retroperitoneal nodes. The nature of this localized effect may now be considered.

Considerable evidence has accumulated to show that foreign protein introduced parenterally into the body induces proliferative activity in the lymph nodes to which it is carried by the lymph stream. This is familiar in the swelling of the regional lymph nodes folowing localized infection. The experimental evidence has been reviewed by Drinker and Yoffey ('41). These authors cite work which shows that small amounts of unchanged protein are normally absorbed without being broken down by the digestive enzymes. These considerations suggested to us that at least a part of this foreign protein from the intestinal tract may normally enter the intestinal lymph and so reach the mesenteric lymph node which may react by increasing its size while taking up or inactivating the protein. Now if this node be removed, the foreign protein in the lymph first comes into contact with lymphoid tissue in the retroperitoneal nodes which had previously been protected from such contact by the mesenteric node. With removal of such protection the protein would exert its influence by increasing the weight of the retroperitoneal nodes.

The general hypothesis may be suggested that the lymph arising in tissues contains substances, possibly proteins, which are taken up by the first lymph node in the course of the stream and so maintain the size of the node. However, if this node be removed, the substance may reach a second more central node and there exert an influence increasing the size of this second node.

A hypothesis has been suggested by Rouvière and Valette ('37) which relates the size of lymph nodes to the regional lymph flow. They observed that following extirpation of the popliteal node of the rabbit, the degree of regeneration of that node was proportional to the number and size of its afferent lymph vessels. They suggest that "Le volumne des ganglion est adapté aux fonctions que ceux-ci remplissent et il doit être suffisant pour assurer l'accomplissiment de ses fonctions . . ." This hypothesis becomes identical with ours if the assumption is made that it is not the flow itself but certain substances contained in the flow which exert this regulatory function on node size.

To test this hypothesis a second series of experiments was undertaken. According to Job ('15) the drainage from the "intestinal" (mesenteric) nodes of the rat passes to the "cisterna group of lymph nodes" (which appear to be homologous with the retroperitoneal nodes of the mouse) and does not reach the "lumbar" (iliac) nodes. In eight young female albino rats, the mesenteric nodes were removed; 10 days later the animals were sacrificed and the weights of all the remaining nodes were determined. The method was that previously described for the mouse. Eight litter mate control animals were not subjected to any operation but were necropsied and studied similarly. The results appear in table 2.

TABLE 2

Effect of removal of the mesenteric nodes in the rat and mouse.

	RAT		MOUSE	
	Control	Operate	Control	Operate
No. of animals	8	8	10	10
Mean age in days	62	63	256	258
Mean body wt. in grams	119.84	115.90	21.50	21.60
Wt. in mg.				
Iliac				
Mean	20.33	19.38	4.00	5.75
St. Dev.	± 6.32	± 10.85	± 2.39	± 3.00
Retroperitoneal				
Mean	30.56	45.63	6.10	10.35
St. Dev.	± 6.94	+ 20.98	± 2.47	± 5.26
Wt. in mg. of all nodes excluding iliac and retro.				
Mean	214.60	216.60	39.50	38.45
Wt. in mg.				
Thymus				
Mean	230.20	240.50	35.20	29.80

After the removal of the mesenteric nodes, the retroperitoneal nodes of the operate group showed an increase in weight, averaging 16% ($P = 0.07$). This change, along with the fact that there was no increase in weight in the other lymph nodes weighed, lends support to our hypothesis.

Since no data on the drainage channels of the mouse were available, and in order to obtain some evidence of the drainage of lymph from the mesenteric node of this animal, india ink (0.1 cc.) was injected into the mesenteric node. At the end of 1 week, the abdominal cavity was opened. Particles of india ink were observed in the iliac nodes; at the end of 2 weeks they were found in the retroperitoneal nodes. With this evidence of lymph drainage pathways from the mesenteric node in the mouse, experiments identical with those on the rat were carried out on ten adult female albino mice. The results also appear in table 2. It is evident that removal of the mesenteric node had produced a considerable and significant increase in the size of the retroperitoneal nodes ($P = 0.05$) and a possible increase in the iliac nodes ($P = 0.10$). The weights of the rest of the nodes studied did not change. These results in the mouse also support our hypothesis. The fact that the similar results have been obtained in both the rat and the mouse indicates that the phenomenon is not specific for either species.

The mesenteric lymph node which was removed from the mouse had an average weight of 20 mg., the increase in the mean weights of the retroperitoneal nodes was 4.3 mg. and that of the iliac nodes 1.8 mg., a total of 6.1 mg. Thus the increase was only about one-third of the weight of the excised tissue. In this connection the findings of Rouvière and Valette ('37) may be cited. They showed in the rabbit that if the lymph vessels around a partially resected lymph node reform so as to detour the lymph stream away from that node, it may atrophy completely. If in our experiments the regeneration of the lymphatic vessels in the operated region conducted the lymph through channels which did not include either the iliac or retroperitoneal nodes, the relatively incomplete restoration of lymphoid tissue mass may be explained.

We may now consider the bearing that this demonstration of the regional factor controlling lymph node size may have on the interpretation of those of our experiments in which a large proportion of the lymphoid system was removed. Of the four groups of test nodes, the regional factor is clearly operative in the case of the retroperitoneal and iliac groups. It may also play a role in the case of the deep cervical nodes, since the superficial cervical nodes, which were removed, may normally drain into them. The popliteal nodes are the only ones of the test nodes clearly not subject to a regional influence and are the only group which fail to show any increase in size when a considerable portion of the lymphoid system is removed. In addition to the popliteal nodes the thymus may be added as a lymphoid organ not subject to the regional influence. Its weight was not increased after operation. There thus appears to be no evidence in our experiments for a general systemic factor which might induce a compensatory increase in the weight of lymphoid tissue remaining in the body when most of the nodes have been removed. In view of the findings of Wiseman ('31), Bracco ('39), Jordan and Robeson ('42) and Sanders and Florey ('40) which have been discussed in the introduction of this paper, we do not feel justified in denying that such a systemic factor may exist.

SUMMARY

After removal of approximately one-half of the organized lymphoid tissue in the normal white mouse, an increase of weight of the remaining lymph nodes is observed 10 days after operation. This amounts to 46% of their original weight. The increase does not occur uniformly throughout the lymphoid tissue remaining in situ, but is confined to certain regions, namely those nodes lying central to the excised tissue.

After removal of the mesenteric node in mice and rats, the nodes into which the lymph from the mesenteric node normally flows, increase in size.

These data suggest the hypothesis that some substance in the lymph reaching a lymph node normally maintains the size of that node. The substance being inactivated in this node cannot then influence the growth of the more centrally situated nodes. If, however, the more peripheral node be removed, the substance then causes increase in weight of the more central nodes. The enlargement of the regional nodes which follows infection of peripheral areas may be conceived as being an exaggeration of this process, which operates normally in regulating the size of the lymph nodes.

There are data in the literature which suggest that extensive removal of lymphoid tissue may lead to a (general) systemic factor operating to increase the lymphoid tissue throughout the body. Our results lend no support to this concept.

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EXTRA-EPIDERMAL AND SUPERNUMERARY LENSES IN ASSOCIATION WITH CYCLOPEAN EYES IN AMBLYSTOMA EMBRYOS

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ONE PLATE (FOUR FIGURES)

INTRODUCTION

The question of lens formation under varying experimental conditions, has long been a subject of investigation. Aside from the conditions under which the epidermal ectoderm is induced to form lenses, the formation of these bodies from other sources, namely the material of the optic cup, has been intensively studied. All of the determinations from these investigations have been formulated on the basis of regeneration experiments.

The original observations of Wolff and of Colucci showing that the dorsal rim of the iris could, under certain conditions, give rise to lenses, led to much experimentation. In the course of these investigations, it was found that not only could the dorsal rim of the iris give rise to lenses, when the original lens had been extirpated, but that various parts of the retina also possessed this power. In fact, Fischel ('00, '02) showed that this could occur in both undifferentiated retinas and in the ganglion cells of differentiated retinas. It was also found that, when a fragment of the lens remained, or when a fragment was reintroduced, both the fragment and the iris or retina would form lenses which often united.

Adelmann ('28) showed that similar phenomena resulted when optic fragments of Amblystoma embryos were implanted into heterotopic positions. In a number of cases, he found that two lenses were present, one arising from the dorsal rim of the iris, and the other, which was larger and further developed, from an undetermined source. Similar results were obtained by Alexander ('32) in chicks.

It is to be remembered that all of these experiments were investigations involving regeneration phenomena. In the observations here reported, regeneration, in its strict sense, is not involved. Here, in a series of experiments performed with the view toward examining certain questions involving cyclopia, some rather striking phenomena regarding lens formations, and lens relationships, were observed.

MATERIALS AND METHODS

Amblystoma punctatum embryos, collected in the Chicago area, were used. The operations were performed during Harrison's stage 14, the wide open neural fold stage. The method used by Adelmann ('34) was followed. It consists of cutting along the lower edge of the transverse fold, freeing the anterior part of the neural plate, and lifting this as a flap. The exposed roof of the archenteron was then removed. The flap was allowed to fall back into place and heal.

Control operations consisted of the same procedure, except that the roof of the archenteron was not removed.

The embryos and larvae were fixed in Bouin's solution, dehydrated, embedded in paraffin, and cut at 10 micra. The sections were stained with Harris' haematoxylin. A counterstain was not used.

RESULTS

Lenses which apparently arose from the dorsal rim of the iris, were found in various stages of development. In all of these cases, the continuity between lens and iris is such, that not only are the nuclei oriented in the same plane, but there is a progressive transition of nuclear shapes. The nuclei of the iris and the retina are elongate, while those of the periphery of the lens are imperfectly spheroidal. Where lens and iris unite, nuclei transitional between these two extremes of shape occur.

A specimen, fixed during stage 34, illustrates a very early stage in the formation of this type of lens (fig. 1). Here, the iris takes on a spiral appearance, and the nuclei within the spiral are assuming a shape characteristic of those found in the periphery of the lens; the imperfect spheroidal shape. The beginnings of a cavity may be seen, but lens fibers are absent. The picture here has a very close resemblance to the illustration given by Sato ('30) showing lens formation from the dorsal rim of the iris in Triton.

An illustration of a later stage in the development of such lenses is found in a larva which was fixed during stage 39. This specimen has a well formed lens, about 110 micra in diameter, which has begun to produce fibers. Its peripheral nuclei lie in the same plane with those of the iris. In addition, the progressive transition of nuclear shapes, from the imperfectly spheroidal of the lens to the elongate of the retina, is present.

Of a total of fifty lenses observed, ten showed these relationships. There does not appear to be any correlation between this type of lens formation, and the side on which it is formed. Six of these ten lenses were formed on the left side, and four on the right.

It should be pointed out that these lenses are not the products of regeneration per se, for the operations were performed before there was any visible morphological evidence of a lens. However, Carpenter ('37) has shown that the lenses in A. punctatum are determined very early, and it may well be that the operation destroyed the anlage, with consequent formation of a lens from the rim of the iris. The formation of lenses in this manner, however, cannot be regarded as compensatory, for, as will be shown below, four of the ten cases cited, have lenses in addition to those discussed. The other lenses are not of iris origin.

Six cases have been found among the fifty lenses examined, in which a lens apparently originates from the retina. The association between these lenses and the retina is similar to that found between the iris and the lenses originating from this source. At the point of apposition between retina and lens, the nuclei of both are orientated in the same plane, and the transitional shapes from the elongate of the retina to the imperfect spheroidal of the lens, are found (fig. 2).

AP 36, fixed during stage 39, illustrates a case in which the lens rests against both the retina and the epidermis. In the former relationship, there is a common orientation of the nuclei, and the transition in shape between the two. In the latter, there is a distinct demarcation between the epidermis and the lens. It is apposition without fusion.

AP 16, fixed during stage 40, illustrates a case in which the retina forms a pocket to receive the lens, and remains distinct from it, except for an interval of about 30 micra, in which the two are united in the same manner described for AP 36.

In two of the six cases, supernumerary lenses are found. Thus, again the question of formation of a lens in this manner cannot be considered a compensatory phenomenon.

The possibility should be considered that these lenses originated in the usual manner from the inner layer of the epidermis, detached, migrated, and fused with the retina. It is doubtful that this occurred, for all of these lenses are in stages of development in which retention to the source of origin should be maintained. The lenses are developing fibers, but are not completely differentiated. In addition, they are of the same size range as those found originating from the rim of the iris. Thus this possibility is not likely, for at no time has it been observed that any lenses of similar developmental stages, regardless of the source of origin, have detached from their source and migrated to other positions. Fischel ('00, '02) and Adelmann ('28) have shown that lenses may well originate from the retina.

In addition to these two sources of origin, twenty-seven of the fifty lenses examined, originated from the nervous layer of the epidermis. In each case, the lens is seen to retain its connection with its origin. These lenses range from placodes to bodies which are fully differentiated, and exhibit good fiber formation.

It has been stated above, that those lenses which were formed from either the dorsal rim of the iris, or from some part of the retina, could not be regarded as compensatory products. That is, they could not be regarded, in the strict sense, as products of regeneration due to the absence or removal of a previously formed lens. In eight of the forty-three specimens, two or more lenses were present in a single eye. Five of these eight had two lenses, two had three lenses, and one had four lenses. These supernumerary lenses are of varying origin.

In some cases, more than one lens may be formed from a single epidermal placode. In other cases, two separate epidermal placodes are involved. Two specimens, AP 7 fixed during stage 41 and AP 11 fixed during stage 39 illustrate the former possibility. In both of these specimens, a very young placode has a dorso-ventral cleft which would indicate the formation of two lenses. AP 16, fixed during stage 40, shows a late stage in this type of double lens formation. Here two well differentiated lenses are united to each other and to the epidermis.

A still later case is illustrated by an instance of spontaneous cyclopia discovered among a group of larvae. This specimen was fixed during stage 44–45, and exhibits a typical cyclopean eye, in which there are two lenses in an advanced stage of differentiation. These lenses are united to form a dumb-bell shaped body. The origin of these lenses is uncertain, for they show connection neither with the epidermis nor with any part of the optic cup. However, from the observations on the experimentally produced cyclopias, it may be considered that these lenses are of epidermal origin, from a common cleft placode.

AP 36, fixed during stage 39, has three lenses of epidermal origin, but each of these lenses appears to have originated from an individual placode, and is far removed from the others. None of these lenses is united to any of the others.

AP 2, fixed during stage 36, exhibits a similar case in which two lenses are of epidermal origin and quite separate from each other, while a third placode shows a cleft which may indicate the formation of two lenses as described above.

In addition to the supernumerary epidermal lenses, other associations have been found. As has been noted above, AP 16 had two epidermal lenses originating from a common cleft placode. Added to this, there is a lens originating from the retina. This lens is well-formed, shows fiber formation, and is rather large: 100 micra in diameter. Specimen AP 22, fixed during stage 40, exhibits four well defined lenses (see Plate 1, figs. 3, 4). One is of iris origin, one of retinal origin, and two are of epidermal origin, arising from separate placodes. One of the epidermal lenses, and the lens which originates from the iris, appear to be of a similar size and stage of development. The retinal lens is smaller, not as well differentiated, and the transitional nuclei discussed above can be seen clearly. The other epidermal lens is quite small, and could more properly be called a lentoid body.

The relations among the supernumerary lenses are not constant. They may have any combination of origins and of stages of development, as has been shown above.

Six cases exist in this series, in which no lens is associated with the eye. In one specimen, fixed during stage 42, two eyes were formed, but one is much reduced. It is invested heavily with a pigmented tapetum and is far removed from the epidermis: it has no lens. The other eye is normal, and has a normal lens. In other cases, the eyes have no lenses, regardless of the stage of development. These eyes are apt to be buried in the ventral yolk during the earlier stages. They are far removed from the epidermis.

In the operated controls, in which the operation was performed to the extent of cutting along the margin of the neural plate, lifting the flap, and allowing it to fall back into place and heal, seventeen of thirty-five lenses were of epidermal origin; two and possibly eight were of iris origin; and eight exhibited a total lack of lenses. There were no cases of lenses originating from the retina, and there was no instance of multiple lens formation. The latter result was to be expected, on the basis of the hypothesis regarding the formation of supernumerary lenses.

DISCUSSION

The subject of formation of lenses from the dorsal rim of the iris has long been a subject of contention. Only in Triton, Triturus, and Salamandra, can it be said to have definitely occurred, according to Stone and his co-workers. They believe that most of the previous work on this subject has, in recent years, been shown to be faulty or misinterpreted. Stone and Sapir ('40) report that they have failed to find any signs of Wolffian regeneration in five species of amphibians in which regeneration of lenses from the dorsal rim of the iris had previously been recorded. In 181 experiments on Rana clamitans, R. pipiens, Amblystoma punctatum and A. tigrinum, they failed to find any signs of lens formation from the dorsal rim of the iris. In 330 previous experiments on A. punctatum, Stone and Dinnean ('40) reported that they had obtained the same negative results.

Beckwith ('27) reported that in 25% of ninety-four cases of operations on A. punctatum, in which the operations were performed during stage 20–21, she obtained regenerating lenses from the dorsal rim of the iris. Stone and Sapir ('40) conclude, from a study of her figures, that she presented only one case in which this phenomenon is present.

Stone and Chace ('41) reported that they had obtained Wolffian regeneration in Triturus viridescens. They believe that in this and in Triton taeniatus and possibly in Salamandra, the claims are substantiated.

The experiments performed by these workers were performed on specimens which either had functional eyes, or in which the neural folds had already closed. Thus, definitive lenses, or lens placodes were extirpated so that a regenerative process was involved, in which a new lens was formed to compensate for the loss of an old one. Although the process of regeneration may be regarded as embryological, the conditions here reported are not quite analagous to those of the experiments quoted above, for here lens placodes were not yet formed at the time of operation.

It may be, however, that presumptive lens tissue was injured to the extent that it was unable to regenerate, for Carpenter ('37) and Vogt ('29) showed, by staining experiments, that the presumptive lens areas in the medullary plate stage, lie, on both sides, at the border of the neural fold near the anterior end of the embryo. Thus, at the operation, the lens anlage would of necessity be injured. Furthermore, Spemann ('38) and Carpenter ('37) point out that in A. punctatum the lenses are determined very early in the developmental history of the animal. Such conditions would give rise to a situation comparable to those involved in regeneration, and could result in the absence of lenses, or in lenses being formed from extra-epidermal origins.

It could be assumed that the injury was equal on both sides, as a result of the operation, and thus, both lenses, if more than one were present, would arise from the rim of the iris, or from some other source. That this occurs, can be seen in the cases cited in which supernumerary lenses were present. Furthermore, it appears that lenses were formed from the iris with equal frequency from both sides.

As to the specific causes involved in the formation of lenses from the dorsal rim of the iris, little can be said. Wachs ('14) postulated that Wolffian regeneration was due to the removal of a lens, for the lens present produced a secretion which inhibited the retina from stimulating the iris to produce a lens. Sato ('30, '33), on the other hand, showed that Wolffian regeneration of lenses occurs only from that iris rim which is opposite the choroid fissure. Beckwith ('27) also showed that there was some relation to the position of the fissure. Neither of these postulates apply to the cases here presented, for these lenses are apparently formed in the presence of other lenses, and since there is no optic stalk in cyclopia, there can be no choroid fissure to exert an influence.

Thus neither the formation of lenses from the dorsal rim of the iris, nor their formation from the retina can be explained by any of the existing hypotheses. The species in which such formations have been verified, may also need further investigation.

The inability of the lens anlage to form lenses after the operations, in both the experimental series and the control series, would indicate that the lens anlage may have been injured. The cases cited in which there is no lens associated with an eye, are points in evidence.

However, the phenomenon of supernumerary lenses remains unexplained. Stockard ('10) reported such conditions in cyclopean eyes resulting from the action of various inhibiting agents, but did not discuss them. Adelmann ('28) found cases in which two lenses were associated with each heterotopically transplanted eye. Neither the origin of the extra lens, nor the reason for its presence, was determined.

SUMMARY

1. In Amblystoma punctatum, a series of operations was performed during Harrison's stage 14, in which the anterior end of the medullary plate was lifted as a flap, the roof of the archenteron removed, and the flap allowed to fall back into place and heal.

2. Controls were established by performing the entire operation without removing the roof of the archenteron.

3. A total of fifty lenses in the experimental specimens, and thirty-five in the controls, was observed.

4. In the experimental specimens, ten lenses originated from the dorsal rim of the iris, six from the retina, and the remainder from the inner layer of the epidermis.

5. In the control series, two and possibly eight lenses originated from the dorsal rim of the iris, and the remainder from the inner layer of the epidermis.

6. No cases were found among the controls in which a lens originated from the retina.

7. Four cases, in the experimental series, were found in which supernumerary lenses were present. Two of the eyes had three lenses and one had four lenses. None of the controls exhibited this phenomenon.

8. Six cases among the experimental specimens had eyes for which there was no lens. No cases were found in which a lens was not associated with an eye.

9. Lenses were found for all of the eyes in the control series.

ACKNOWLEDGMENT

I would like to express my gratitude to Dr. Graham P. DuShane for suggesting the problem, and for his aid during its investigation.

This represents a portion of a thesis submitted to the faculty of the Division of Biological Sciences in candidacy for the degree of Master of Science in the Department of Zoology, the University of Chicago.

It was my intention to round out this investigation by further experimental work before publication. However, my entrance into the Army of the United States precludes further work for an indefinite period.

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PLATE 1

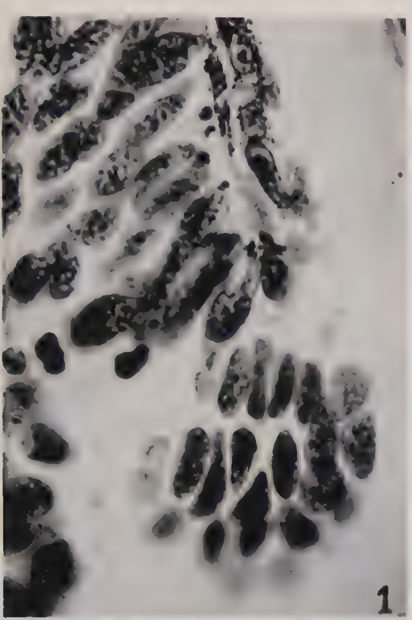
EXPLANATION OF FIGURES

1 Showing continuity between lens and dorsal rim of the iris of a specimen fixed during stage 34. The nuclei of the central portion of the lens (L), are beginning to assume the spheroidal shape. × 550.

2 Specimen AP 22, fixed during stage 40 showing continuity between lens and retina. This is L 3 of figure 3. × 540.

3 Same specimen as in figure 2 showing three of its four lenses, L 1, L 2, L 3. × 100.

4 Same specimen as in figures 2 and 3, showing the fourth lens whose origin is from the epidermis. Lens 1 and lens 2 may still be seen. × 100.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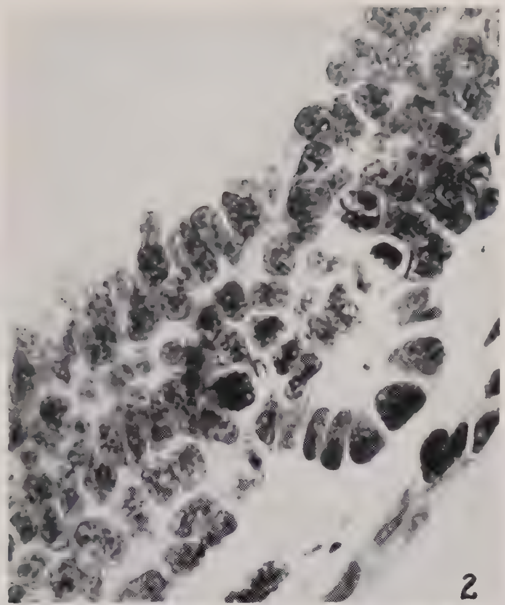
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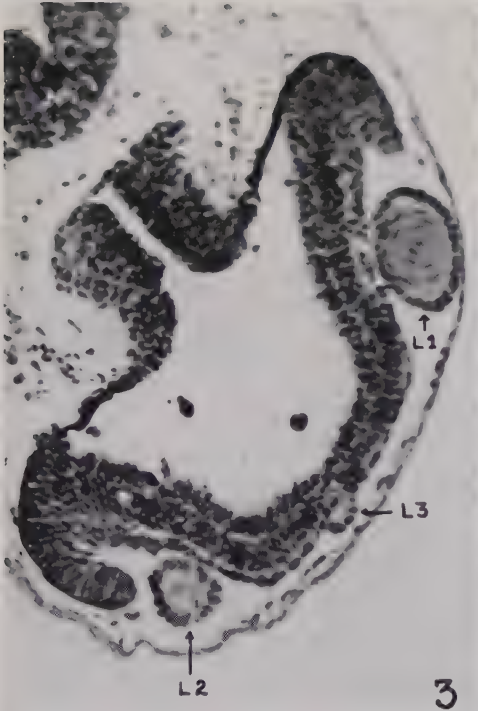

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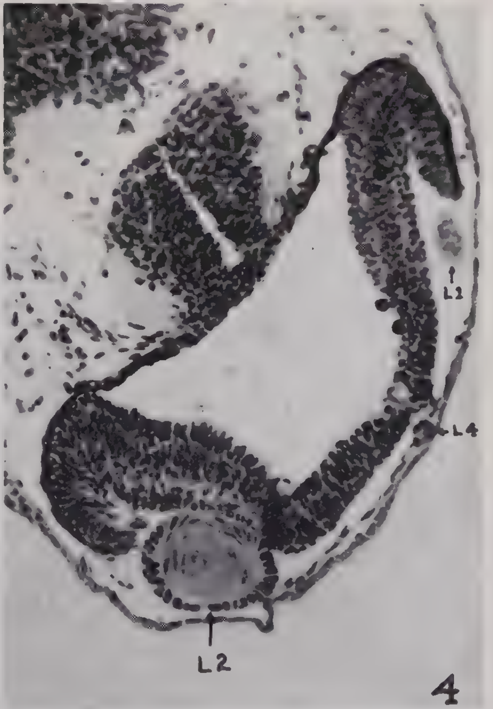

4

THE LYMPHATICS OF THE PARIETAL TUNICA VAGINALIS PROPRIA OF MAN

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ONE TEXT FIGURE AND ONE PLATE (THREE FIGURES)

INTRODUCTION

The lymphatic plexuses of the parietal tunica vaginalis have not been described previously. There are perhaps two reasons for this gap in our knowledge of the lymphatic system. First, there are no lymphatic plexuses in the tunica vaginalis of the common laboratory animals. Second, they are frequently difficult to inject, either with the needle or by employing the usual technique of injecting subserous lymphatics, i.e. by placing a suspension upon the serous membrane and imitating the normal movements of the tissue.

MATERIAL AND METHODS

The material was secured at autopsy within a few hours after death, the spermatic cord and entire contents of the scrotum being shelled out intact. The saccus vaginalis was entered at its lower extremity through a small incision. A needle was inserted and the cavity was flushed out repeatedly with normal saline to remove all traces of serous fluid which might cause clumping of ink particles and so prevent their absorption. Five cubic centimeters of India ink were injected and the incision closed with a hemostat. The entire preparation was massaged with the fingers for about 5 minutes. The sac was then opened widely and all traces of ink were washed out with normal saline.

This technique sufficed to inject the subserous plexuses but not their efferent channels. Attempts were made to enter the subserous plexus in order to inject the entire efferent drainage. This technique was successful in two of twelve specimens.

All preparations were fixed in 10% formalin. Portions of the parietal tunica vaginalis were prepared as whole mounts in glycerin. Other portions were double embedded, sectioned, and stained with hematoxylin and eosin.

OBSERVATIONS

Following the method of massage described above one can see clearly with the naked eye the subserous lymphatics filled with absorbed ink. The subserous lymphatics do not form one continuous plexus throughout the entire extent of the parietal tunic but occur rather in patches which average about 5 mm. in diameter (fig. 2). These patches are larger and more numerous over the anterior portion of the sac. There are no lymphatic plexuses in the visceral tunica vaginalis propria concerned with absorption from the saccus vaginalis.

The subserous lymphatics consist of superficial and deep plexuses (figs. 1, 2, and 4), both being confined to the basal fibrous layer of the tunica vaginalis. The vessels which make up the superficial plexus are much smaller and their endothelium is intimately related to the mesothelial lining of the sac. The superficial plexus communicates by narrow channels with the deep plexus which lies within the deeper portion of the basal fibrous layer. In sections (fig. 4) the deep plexus appears as irregular excavations in the deeper fibrous framework of the parietal tunica propria. Both superficial and deep plexuses (fig. 2) lack the striking symmetry of the subserous lymphatic plexuses of the diaphragm (MacCallum, '03; Allen, '36; Allen and Vogt, '37).

Figures 1 and 3 show the general arrangement of the efferent drainage of the subserous plexuses. Each subserous plexus is drained by short efferent channels which unite to form one or two large efferent lymphatics which course around

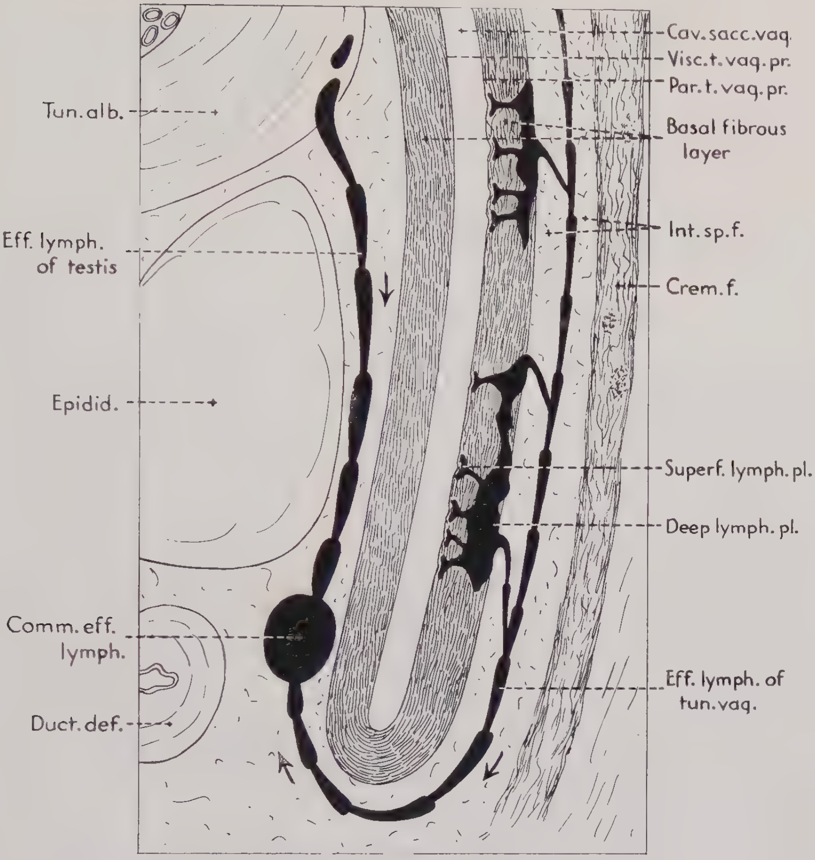
Tun. alb.
Eff. lymph. of testis
Epidid.
Comm. eff. lymph.
Duct. def.
Cav. sacc. vag.
Visc. t. vag. pr.
Par. t. vag. pr.
Basal fibrous layer
Int. sp. f.
Crem. f.
Superf. lymph. pl.
Deep lymph. pl.
Eff. lymph. of tun. vag.

Fig. 1 Section of testis and saccus vaginalis showing deep and superficial lymphatic plexuses which occur in the basal fibrous layer of the parietal tunica vaginalis propria. The efferent lymphatics of the tunica vaginalis course around the parietal tunica vaginalis propria from the medial to the lateral side and enter a common efferent lymphatic which also receives efferent lymphatics of the testis.

the tunica vaginalis from the medial to the lateral side and empty into a large marginal vessel which originates within the testis.

DISCUSSION

It should be noted that the lymphatic plexuses concerned with absorption from the saccus vaginalis lie in the parietal tunic and that there are no absorbing lymphatic plexuses in the visceral tunica vaginalis. The lymphatics which are so easily demonstrated in the latter location are the efferent channels of the lymphatic plexuses of the parenchyma of the testis. It seems to be a general rule that the lymphatic plexuses concerned with absorption from serous cavities are always situated within particular areas of parietal peritoneum which are subject to rhythmic movements and are never situated within visceral peritoneum.

The method of injection and the appearance of the lymphatic plexuses in section make it plain that these vessels are essentially similar in function and structure to the subserous lymphatics of the diaphragm, costal pleura, and pericardium, and that absorption is of the intercellular stomatal type (Allen, '36; Allen and Vogt, '37). The difficulty which we experienced in obtaining in the same preparation a complete injection of all the lymphatic "patches" and their entire efferent drainage indicates that we were unable to imitate by massage the exact muscular movements which in life must account for the lymphatic absorption and flow of the normally occurring serous fluid of the sac. The alternate changes in lymphatic volume which bring about absorption and flow are most probably effected by the vermicular movements of the dartos, aided possibly by the cremaster muscle.

Before the difficult needle injections were successful the possibility was considered that the processus vaginalis might have carried its own lymphatic plexus in its descent into the scrotum. It is now apparent that the lymphatics of the parietal tunica vaginalis propria possess the same regional lymph nodes as the lymphatics of the testis, one or more of the abdomino-aortic group (Rouviere, '36), and that lymphatic invasion of the parietal tunic must take place after the testis has established its definitive relation to the processus vaginalis, a relatively late development.

According to Symington ('14), man is the only animal which possesses a saccus vaginalis which does not communicate with the general peritoneal cavity. This he considers an adaptation to the erect posture. In so far as we have been able to determine, the common laboratory animals whose vaginal processes remain patent throughout life, do not possess a set of lymphatic vessels comparable to those of the parietal tunica vaginalis of man.[1]

There are then two developmental factors in the processus vaginalis peculiar to man: obliteration of the proximal processus vaginalis and establishment of a lymphatic absorptive apparatus. Formation of serous fluid is common to man and animals, but only in the latter can fluid escape through the patent processus vaginalis. It is theoretically possible that obliteration of the proximal processus vaginalis before the establishment of an effective absorptive system would allow serous fluid to accumulate in the sac and result in congenital hydrocele of the newborn. On the other hand accumulation of fluid at a critical time might prevent obliteration of the proximal vaginal process and predispose to congenital hernia.

The author advances the hypothesis that there is an interrelationship between the embryologically late establishment of the lymphatic drainage system of the saccus vaginalis and the late and variable (Mitchell, '39) obliteration of the proximal portion of the processus vaginalis which may explain the two most frequent congenital defects of this region.

SUMMARY

The parietal tunica vaginalis of man possesses a lymphatic plexus comparable to the subserous plexuses of the diaphragm and intercostal pleura. This plexus drains into the same regional lymph nodes as the lymphatics of the parenchyma of the testis.

Development of a lymphatic plexus in the parietal tunica vaginalis, like obliteration of the proximal portion of the processus vaginalis, is peculiar to man. An hypothesis is advanced in this connection in regard to the most frequent congenital defects of the processus vaginalis.

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[1] These include one specimen of Macacus rhesus.

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PLATE 1

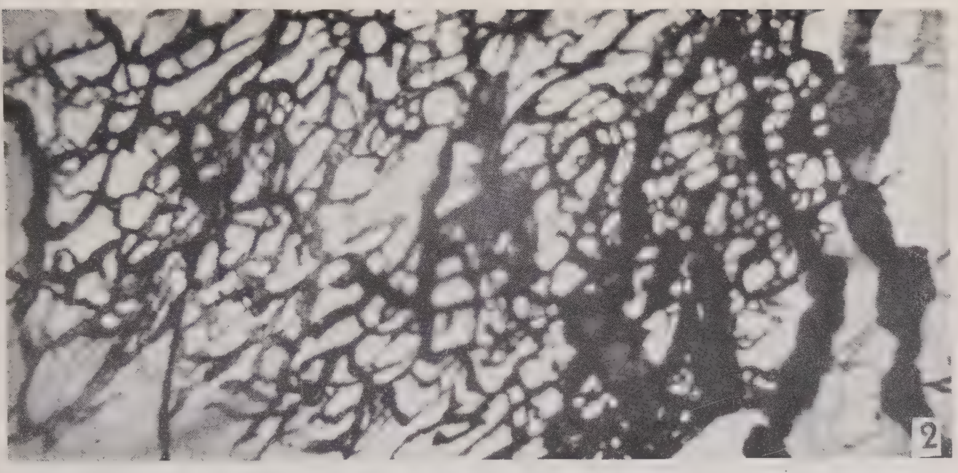
EXPLANATION OF FIGURES

2 A whole mount showing superficial and deep plexuses of parietal tunica vaginalis propria. India ink injection. This plexus is 5 mm. in diameter.

3 A whole mount showing a large efferent lymphatic channel draining three subserous ''patches.'' Indian ink injection.

4 A subserous lymphatic plexus in section. India ink injection. Note that both superficial and deep plexuses are confined to the basal fibrous layer.

S.l.pl., superficial lymphatic plexus; D.l.pl., deep lymphatic plexus; B.f.l., basal fibrous layer; I.s.f., internal spermatic fascia; Cr.f., cremaster fascia.



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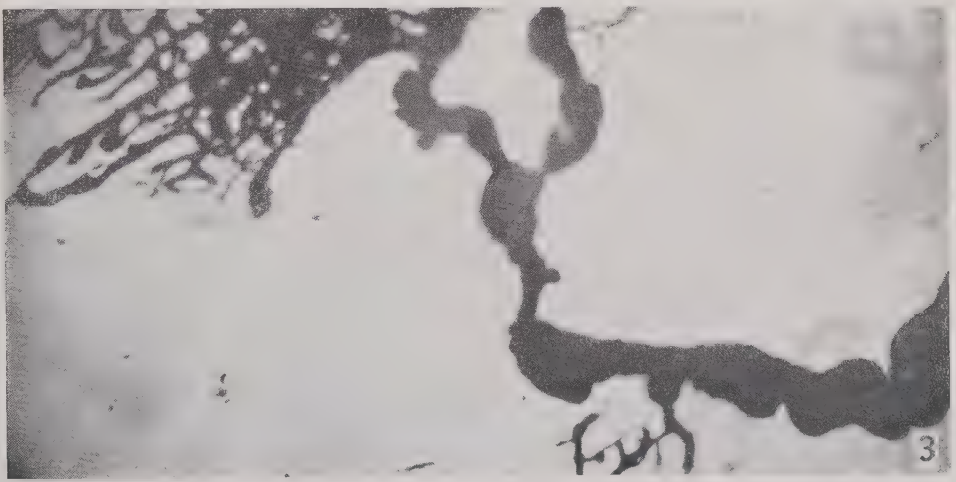

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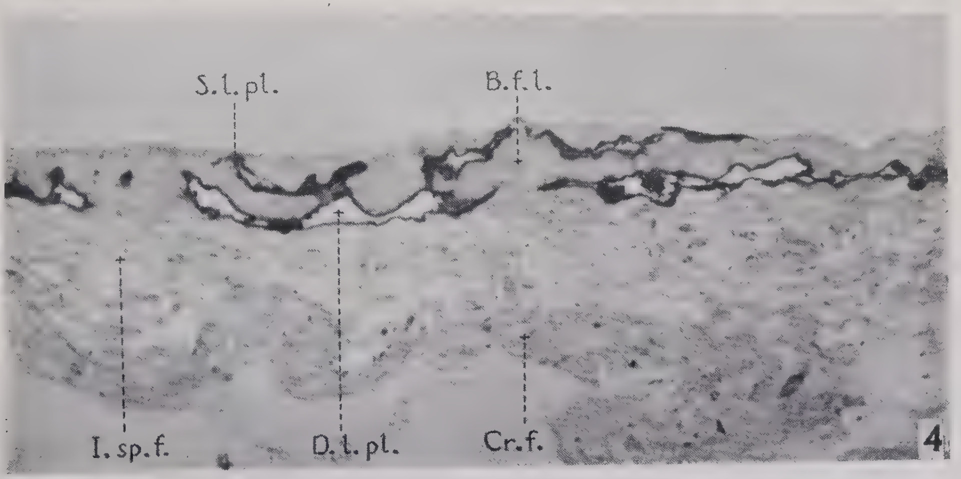

S.l.pl.
B.f.l.
I.sp.f.
D.l.pl.
Cr.f.

4

BOOK REVIEW

PALMAR AND PLANTAR DERMATOGLYPHICS IN PRIMATES. By Charles Midlo and Harold Cummins. The American Anatomical Memoirs, no. 20. The Wistar Institute of Anatomy and Biology, Philadelphia. III + 198 pp. 1942. ($3.00.)

The systematic and thorough interpretation of man as a primate is progressing slowly. We are still unable to answer fully the basic question: What are all the physical characters by which man differs from his nearest animal relations, the non-human primates? The final determination of man's exact place among the primates will have to take into consideration all the structures of our body and their precise degrees of resemblance to the corresponding structures in the vast variety of apes and monkeys. So far this fascinating problem can be solved in only tentative fashion. Comprehensive, comparative studies on primates frequently require more material than is at the disposal of most investigators and many generalizations in the literature are premature, being based upon all too few observations. Among the primates are numerous types which, regarding some structures, vary individually far more than does man, hence many specimens are needed for finding the really representative, average conditions. The manifold potentialities for different combinations in the detailed variations of dermatoglyphics are well known for man. Since the ridge-bearing skin areas can be fully as variable in at least some of the other primates, extensive material is specially necessary in comparative studies on these conditions.

For the monograph under review the authors have been able to study adequate series of specimens. Exclusive of the human series, the material examined consists of 478 hands and 440 feet. An additional 75 hands and 109 feet, illustrated in the literature, have been made use of. Thirty-six different genera, representing all existing families and subfamilies of primates, are included in these records. The observations are confined to palm and sole and to the tails of those New World monkeys with caudal dermatoglyphics. The digits had to be excluded on account of technical difficulties encountered in preserved material.

The first part of the book is devoted to descriptive morphology and deals systematically and in detail with the dermatoglyphic patterns as found in each genus. Here are also shown the general correlations between volar pads and dermatoglyphic configurations. Hundreds of clear and very helpful drawings accompany these discussions and form in themselves an original and very valuable catalogue of primate palms and soles. A second part of this volume approaches the problem with quantitative methods and analyzes the pattern intensity and the interrelations between groups of configurational fields, as well as bilateral asymmetry in these conditions. The last section is of particular interest to the primatologist and to students of human evolution since it discussed at length the affinities among the primates as far as these are indicated by the complex findings on dermatoglyphics. From the final conclusions, as given in the summary, the following is especially noteworthy:

> ''Gibbon is the most specialized simian. The three great apes and man present specializations which follow different directions. While these divergent specializations render comparison difficult, the order of increasing specialization indicated by the pooled evidence is orang, gorilla or chimpanzee, man. Especially in adherence to the basic plan of configurations, man is even more primitive than orang; inasmuch as that plan is so fundamental a characteristic, it is concluded that man stemmed from an ancestral stock more primitive than any recent ape, having dermatoglyphic traits more closely allied to those of monkeys.''

This memoir represents a much needed contribution to our knowledge of primates. Future work on dermatoglyphics will add to, but not replace the well supported results of this scholarly investigation.

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